ON THE GENETICS OF SUBNORMAL DEVELOPMENT OF THE HEAD (OTOCEPHALY) IN THE GUINEA PIG

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Received November 16, 1933

INTRODUCTION

With the solution of the immediate problems of the numerical phenomena of heredity and of the organization of hereditary units in the cell, the center of interest in genetics is tending to shift towards questions of the physiology of gene action. Most of the genetic differences which it has been convenient to study have had to do with rather superficial characters in which the differential activities of the genes probably take place in cells of rather late developmental stages. This has its advantages on the physiological side since in such cases the chain of processes intervening between primary gene action and the observed character difference is likely to be relatively short. On the other hand, it would be of great interest to learn something of the nature of the genetic control of the more basic developmental processes—type of polarity, cleavage, gastrulation and the formation of the embryo. The subnormal development of the head shown by otocephalic monsters seems to involve processes at this level. Such monsters, ranging from types normal except for slight reduction of the mandible or of the premaxilla, to a type in which the head lacks jaws, nose and eyes and the brain terminates in the medulla, have been appearing for years in relatively large numbers in a particular inbred strain[1] of guinea pigs. However poor these may be as material for the study of heredity it has seemed worth while from the standpoint of developmental physiology to learn as much as possible of their genetics. The present paper supplements a previous account (WRIGHT and EATON 1923).

Questions of the genetics of characters (in the broad sense) are conveniently divided into three categories. First is the determination of the ultimate factors, (genetic or environmental) and the evaluation of their relative importance. Second are questions of the time and mode of action of these factors. Third is the task of tracing and explaining the interactions within the organism which intervene between the primary action of the factors and the observed character changes. The present paper is

[1] This strain (family 13) is one of 23 strains maintained by brother-sister mating since 1906 by the Bureau of Animal Industry of the U.S. DEPARTMENT OF AGRICULTURE. The author was in charge of these experiments from 1915 to 1925. Since that time this strain has been maintained at the UNIVERSITY OF CHICAGO.

devoted primarily to the first group of questions but attention will be called to such deductions as can be made immediately with regard to those of the second group. A recent paper (WRIGHT and WAGNER 1934) is devoted primarily to the third group of questions.

GRADES OF DEFECT

Figure 1 gives semi-diagrammatic representations of these grades. "In grade 1, the only obvious defect is more or less reduction of the lower jaw. In grade 2 no mandible can be felt externally. In grade 3 the ears are connected under the throat by bare skin. In grade 4 there is only a single median ear opening on the throat. In grade 5 the mouth and upper incisors are lost. In grade 6 the nostrils fuse. In grade 7, the eyes are in contact below a narrow nasal proboscis or are more or less fused. This fusion is complete in grade 8. The proboscis is lost in grade 9, the eye in grade 10, and the ear opening in grade 11. Two small ears are the only externally visible organs of the head left. In grade 12, the body rounds off in front of the shoulders, with no sign of a head except a single small median external ear" (WRIGHT and EATON 1923). There have been a few aberrant types. In grade A the principal defect is the shortness of the snout and lack of upper

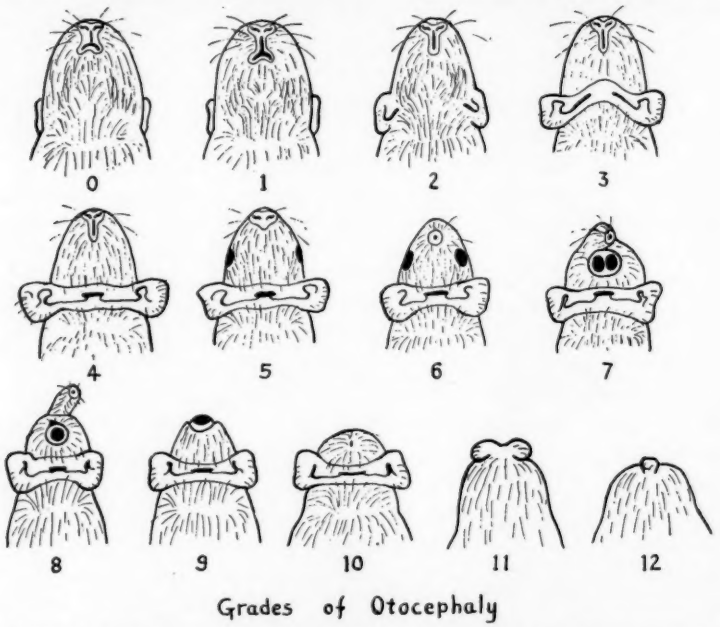

FIGURE 1.—A semidiagrammatic representation of the main series of grades of otocephaly.

incisors. Grade 4A combines this defect with those of grade 4. In grades 8A and 9A, the cyclopian eye appears to be on top of the head, since an upper jaw is present below it. The widest departure from the usual series has been in the case of seven monsters, born since 1925 in the stock of the Bureau of Animal Industry, but which Dr. H. C. McPHEE and Mr. O. N. EATON, who have been in charge, have kindly permitted me to discuss here. In these monsters there are not only no jaws (as in grade 5) but no external ears or ear openings and no external eyes in the small orbits. They are called grade 5C.

The lower grades (1 to 5) belong to the type of monster known as agnathia. In the higher grades, agnathia is combined with gross defect of the brain, manifested externally in grades 7 to 9 by cyclopia. The highest grades (10–12) are of the type known as aprosopus. The series from 3 to 12 and including 8A and 9A but not A, 4A and 5C, agrees well with I. G. ST. HILAIRE's category "otocéphalien," which he distinguished from the closely allied category "cyclocéphalien" in which there is cyclopia without mandibular defect. The term otocephaly will be used as a convenient designation for the whole series.

Animals of grade 1 often live from 4 to 6 days. Two lived longer, 20 and 38 days respectively. On death it was found that the growth of the lower incisors which did not meet the upper incisors, had forced their mouths open. Even the most advanced grades are healthy and nearly normal in size up to the time of birth, following which they die in a few minutes of asphyxiation. One female born since the closing of the tabulation on which the present paper is based lived to an age of 12 months. She differed from normal in having only one (median) lower incisor. Her parents produced two monsters among 12 young; and her sire mated with a sister of her dam produced 1 in 7. This female has had two litters by a half-brother. The 5 young were all normal. Two of the offspring mated with each other produced two monsters to one normal.

OCCURRENCE IN DIFFERENT STRAINS

Otocephalic monsters appear sporadically in many stocks of guinea pigs. In the experiments of the U. S. Bureau of Animal Industry through June 1922 (WRIGHT and EATON 1923), 4 had appeared among 11,000 animals of mixed ancestry. Records were at hand at that time from 24 inbred strains, maintained since 1906 exclusively by brother-sister mating (except for one maintained by parent-offspring mating). Fourteen of these had never produced any monsters of these types (12,000 young). Nine had produced 28 of the monsters in 13,000 young. The one remaining strain (family 13) had produced 50 in 3300, with significant differences among its branches.

These data can now be supplemented by the records of the U. S. Bureau of Animal Industry from July 1922 to December 1925 and of stocks descended from these but bred at the UNIVERSITY OF CHICAGO from 1926 through 1930. The following statements are based on the entire body of data.

Strains with no ancestry of family 13 but which had produced at least one otocephalus yielded a total of 34 in 24,000. Taking into account the considerably larger number of animals from strains which have never produced such monsters, it may be concluded that the ordinary rate of occurrence is less than 0.05 percent. In contrast family 13 has now produced a total of 272 in 6,275 young, or 4.3 percent.

One of the inbred strains which is included above among those which produced no otocephalics during the period of tabulation was family 2. This family has produced some 6,000 young in the stocks of the Bureau of Animal Industry and of the UNIVERSITY OF CHICAGO. As shown later, no otocephalics have appeared in first crosses between it and even the high producing lines of family 13. Yet I am informed by Mr. O. N. EATON who has continued to breed the family in the stocks of the Bureau of Animal Industry, that a group of 160 animals wholly descended from one mating in the 19th generation of brother-sister mating has included seven otocephalics, all of the peculiar earless type described as grade 5C, which has never appeared in any other stock. He informs me that no otocephalics of any sort have appeared in other branches of family 2 in the Bureau stock since 1925, in agreement with the earlier history of the family and with the record of the branches still maintained in Chicago. Mr. EATON informs me that otocephalics have continued to appear freely in the Bureau stock of family 13 since 1925 but these are not included in the present tabulation.

It is clear that inbreeding *per se* does not bring about abnormal development but that special genetic tendencies affecting both frequency and type may occur and be isolated and fixed in particular inbred strains.

The record of family 13 begins with a mating made in 1906 between two animals from the stock of the U. S. Bureau of Animal Industry. The foundation female had previously been mated with another male, but no otocephalics were recorded among 400 inbred descendants. The foundation male was mated with 3 other females. One otocephalus appeared among 2200 inbred descendants. Thus the otocephalic tendency of the original animals does not seem to have been very strong.

The branching lines of descent from this pair, reaching in some cases to the 32nd generation of brother-sister mating, are shown in figure 2. It is convenient to apportion these lines to 18 substrains, each starting from a single mating. The otocephalics are represented by black dots in the figure.

Inspection shows that the incidence of abnormality has been far from uniform. The first case appeared in generation 2 and no others came until generation 8. Three of the substrains which began in the early generations produced no monsters among 199, 196 and 301 young respectively. The 3 other substrains which originated before the 8th generation produced an average of about 1 percent (2 in 151, 4 in 457 and 5 in 629 respectively).

The later representatives of the family fall into two large branches descended from two matings of the 13th generation, with common ancestry

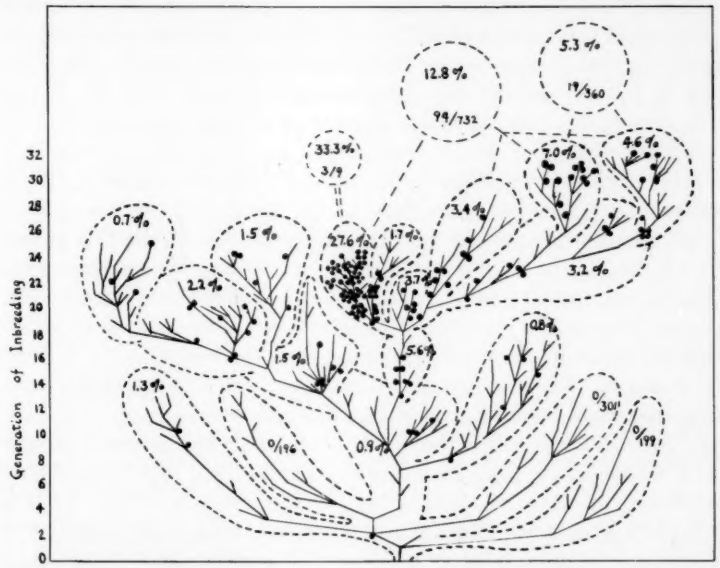

FIGURE 2.—The distribution of otocephalic monsters (black dots) among the branches of family 13, tracing exclusively by brother-sister mating to a single foundation pair. The percentage of monsters in each branch is indicated. The occurrence in three groups descended from crosses within the family are indicated in circles at the top.

4 generations earlier. One of these two branches (descendants of mating 13–13–7) is here divided into 4 substrains ranging from 0.7 percent to 2.2 percent in production of otocephalics (3 in 451, 5 in 325, 6 in 395 and 9 in 408 respectively). These do not differ significantly from each other. Their grand average is 1.5 percent (23 in 1579).

The other large branch (descendants of mating 13-13-1) began at once to produce a distinctly higher percentage of monsters. Excluding the descendants of one mating (13-19-1) to be discussed later, 6 recognized substrains ranged from 3.2 percent to 7.0 percent with no significant differences over a period extending from the 13th to 32nd generation of inbreed-

ing (7 in 124, 7 in 189, 8 in 252, 9 in 264, 13 in 185 and 11 in 240 in order of time). In addition, 19 of the monsters appeared among 360 animals derived wholly from later generations of this group but not from brother-sister matings. These were offspring or descendants of males treated with lead (COLIN 1931). Dr. COLIN'S analysis of this and other data led him to the conclusion that the lead treatment had no demonstrable genetic effect in any respect. Dr. STRANDSKOV (1932) has made a similar study of the effects of X-rays, using the same branch of family 13 as material. While he found a definite reduction in the size of litters produced by X-rayed males, probably indicative of dominant lethal mutations, he concludes that "in no case does the percentage of otocephalics in the progeny of X-rayed males or in their descendants differ significantly from those of contemporary controls." These data are not included in the tabulations of the present paper. It is obvious that there is no significant difference between the 5.3 percent monsters from Dr. Colin's data and the average of 4.4 percent from the 6 substrains referred to above. Combining, we have a group of 1614 animals starting with the offspring of one pair in the 13th generation but ranging to the 32nd generation of brother-sister mating among which 74 otocephalic monsters (4.6 percent) are distributed with remarkable randomness. It is to be noted that the record of this branch as a whole differs significantly (5.2 $\times$ standard error) from the record of the descendants of mating 13-13-7, raised and recorded contemporaneously. Its record differs even more from the early history of the family.

We have so far excluded the record of the most highly producing subbranch of the above high producing branch. Figure 2 brings out the unprecedented incidence of abnormality beginning with a mating (13-19-1) of the 19th generation. This line had produced 17 in 79 (21.5 percent) at the time of the previous paper. Before it became extinct as an inbred strain, through dearth of viable females, it had produced 67 in 355 or 18.9 percent. The detailed record is given in figure 3 and from this it may be seen that there was marked and significant heterogeneity. The original mating produced 2 in 20. One daughter mating produced 7 in 31 and descendants for 4 more generations continued to produce large numbers (44 in 129). The other daughter mating from 13–19–1 produced 2 in 17 and in the next generation two matings produced 6 in 19 and 1 in 3 respectively, clearly demonstrating that the high tendency was present. Yet in the following generation only 2 appeared among 28 young and from this time no more appeared although 99 normals were born. The indication is that the abrupt increase in tendency toward abnormality appeared in one of the pair 13–19–1, in a heterozygous condition, due presumably to a mutation, was transmitted in some lines and lost in others. It may be noted that there is probably heterogeneity even among the matings which

produced monsters. Thus the difference between 2 in 20 and 11 in 26 is 2.4 times its standard error. Among the 9 matings which produced at least 15 young, of which at least 1 was an otocephalus, there is only 1 chance in 20 that the percentages could vary as much as they did by chance (χ^2 test).

The above data from the high producing line may be supplemented by 9 young of which 3 were otocephalic, from matings other than brother-sister, but within the strain. Including these but excluding the substrain in which the high tendency was lost (mating 13-22-5 and descendants) 65 monsters were produced in 234 young or 27.8 percent.

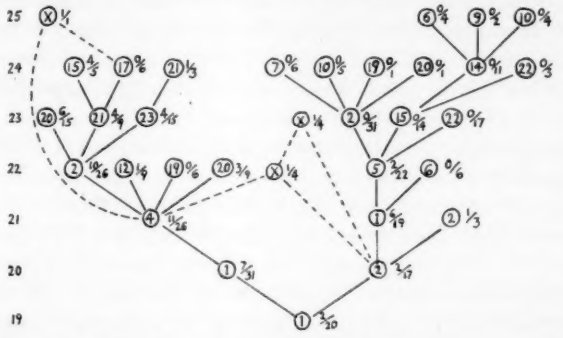

FIGURE 3.—The pure-bred descendants of mating 13-19-1. The number of otocephalic monsters and the total number of young from each mating are indicated in fractional form. All matings were between brother and sister except the three marked X whose connections with their parental matings are shown by broken lines.

A number of animals of this group were mated with animals from the large related group (descended from 13-13-1) averaging 4.6 percent. The total record from such crosses and descendants was 94 in 732, or 12.8 percent, significantly higher than 4.6 percent, and clearly showing that the high tendency was transmitted at least in some cases.

DEDUCTIONS FROM FAMILY HISTORY

Before analyzing further the records of this high producing branch, it will be well to consider what deductions can be made from the rest of the family. The first suggestion is perhaps that the monsters represent Mendelian segregants. The condition, being lethal, cannot of course be a simple dominant. The ratio of normals to monsters in those matings which produced at least one, show that it cannot be a simple recessive. The ratio should be less than 3:1 in data in which only those matings are tabulated which produced at least one recessive. Actually, the 34 monsters recorded from the family, excluding the descendants of 13-13-1, came from 28

matings which produced a total of 485 young. The ratio of normals to monsters is 13.3:1 among matings averaging 17.3 young. Similarly the 74 otocephalics among the descendants of 13-13-1 (excluding those of 13-19-1) were produced by 57 different matings among a total of 812 young. The ratio of normals to monsters is here 10:1 from matings which produced an average of 14.3 young. The 96 matings of this group which produced no monsters had a total of 802 young or an average of only 8.3 per mating, suggesting that their failure to produce monsters was largely if not wholly a matter of chance.

The hypothesis of segregation of one factor being eliminated, it might be urged that the monsters depend on the assemblage of a particular combination of two or more genes, each present in the stock in a heterozygous state. This would be plausible enough if the matings had been made at random within a large stock, but it is ruled out by consideration of the statistical effects of the brother-sister mating actually practiced. Brother-sister mating tends automatically toward the establishment of homozygosis. The number of heterozygous factors in each line should be reduced about 19 percent per generation (in the absence of selection for the heterozygous state). Most lines should soon become homozygous in some combination of the genes present and this combination must of necessity be one yielding fairly normal development. A few lines might become fixed in all but one of the factors for otocephaly and continue to carry this one in a heterozygous condition for several generations. During this period, these matings would produce about 25 percent monsters, but this, as noted, is contrary to the observations. Matings producing monsters in lower frequencies than 25 percent due to heterozygosis in 2 or more factors might be present in the early history but should give way to the 1 factor ratio or to complete fixation of normality. The incidence of monsters through the 32 generations of brother-sister mating is utterly at variance with this expectation.

The possibility that heterozygosis might be retained by a system of balanced lethals was discussed in some detail in the previous paper. It was shown to be untenable from the relatively large size of litter characteristic of family 13 and the absence of any increase in litter size following outcrosses with other stocks. Dr. GEORGE HAINES (unpublished work) has found from an examination of 338 female guinea pigs of the stock of the Bureau of Animal Industry that only 8.7 percent of the corpora lutea were not represented by foetuses and only 7.9 percent were represented by dead foetuses. The average number of corpora lutea per female was 2.59. For 55 females of family 13, included in the above, the average number of corpora lutea was 2.93 of which 14.3 percent were not represented by foetuses and 6.8 percent were represented as dead foetuses. Only 11 of these

females of family 13 had been mated with males of their own family but the record of these was substantially the same: an average of 2.63 corpora lutea, 13.8 percent missing foetuses and 6.9 percent dead. Clearly there is no approach to the 50 percent loss of ova required by the hypothesis of balanced lethals. It can be concluded definitely that the monsters are not Mendelian segregants.

As regards the large group descended from 13-13-1 but excluding 13-19-1 and its descendants, the figures give no evidence that the matings which produced monsters differ from those which did not, or indeed that any mating differed from any other in its tendency. The conclusion which is indicated is that a certain combination of genes had become fixed in this group from its beginning (13th generation of inbreeding), the physiological effect of which is such that each individual has about one chance in 22 (4.6 percent) of falling below the threshold of normality in development of the head. In the group descended from 13-13-7, a constant tendency yielding about 1.5 percent seems to have become fixed. Different tendencies were probably present in the earlier branches but the smaller number of monsters (0.5 percent) make the demonstration uncertain. Even this low tendency, it should be recalled, is at least ten times that among guinea pigs in general.

Even though the monsters cannot be genetic segregants, it is possible that they may differ genetically from their normal brothers and sisters on the hypothesis that each one depends on a separate mutation (using the term in its broadest sense). The particular genetic complex fixed in 13-13-1 may be one which includes an unstable gene or which induces irregular behavior of a particular chromosome.

On this hypothesis the monsters would be distributed at random among the litters of the homozygous line. The same sort of distribution would hold if the genetic complex reacts with non-genetic factors of such an accidental nature as to act wholly on individual embryos.

Most environmental factors would act on litter-mates alike. Malnutrition or disease of the mother, the factors invoked by most teratologists, belong in this category. So also does size of litter. Such factors if effective would *tend* to make litters consist either wholly of monsters or wholly of normals. They would at least cause the occurrence of two or more monsters in the same litter more frequently than from mere chance. Any difference between females of the same stock should also have this effect.

OCCURRENCE IN LITTER-MATES

In order to test this important question, all litters of family 13 have been classified by size and number of monsters. Since different branches of the family differ considerably in mean litter size it is necessary to sub-

divide the family into groups of similar incidence of abnormality. The family has been subdivided for this purpose as follows: Group A includes all of the family except the descendants of 13-13-1 and has an average of 1.0 percent of monsters. Group B includes the descendants of 13-13-1 (except those of 13-19-1) and has an average of 4.6 percent. Group C includes the pure-bred descendants of 13-19-1, except for the low line which originated in 13-22-5. The average is 27.8 percent. Group D includes the descendants of 13-22-5 and cross-bred descendants of a few animals from 13-19-1 which gave no evidence of transmitting the high tendency. The average of this rather heterogeneous group is 3.8 percent. It might well have been included with group B had it not seemed desirable to keep the latter group homogeneous by descent. Group E consists of cross-breds from groups C and B which trace to 13-19-1 through one or more of 4 animals known to transmit the high tendency, but only through one parent. These averaged 10.6 percent. Finally group F consists of cross-breds descended through both parents from the above 4 animals. These averaged 22.0 percent. The data are given in table 1.

The distribution of monsters in litters of each size, expected under random sampling, can be calculated as follows. Let q be the chance of abnormal development in the group in question, $1-q$ be the chance of normal development. In litters of 2 the chance that both will be normal is $(1-q)^2$, that one will be a monster and one normal $2q(1-q)$; and finally that both will be monsters is q^2. In litters of 3 the chances of 0, 1, 2 and 3 monsters are simply the appropriate terms in the expansion of $[(1-q)a+qA]^3$ where a stands for normal and A for abnormal. The expectations for larger litters are given by expansion of the appropriate power of the binomial.

Inspection of the table shows that there is rather close agreement between the observed and the calculated results. The data are combined in a final table, in which the expected numbers are the sums of the expectations in the separate groups (which is not the same as expectations based on the grand average frequency of monsters). Excluding singletons, the number of wholly normal litters is slightly in excess of expectation, of litters with 1 monster only slightly in defect, while litters of 2 or more are again slightly in excess.

	LITTERS OF 2 OR MORE		
NUMBER OF OTOCEPHALICS	OBSERVED	EXPECTED	DIFFERENCE
0	1810	1795.6	+14.4
1	173	191.8	−18.8
2 or more	32	27.6	+4.4

These deviations are in the direction expected if there are factors at work which act on litter-mates simultaneously. But they are so slight that

they might well have arisen by chance. The probability (from χ^2) that as great a system of deviations might arise by chance is .10 (one degree of freedom). It may be concluded that factors which act separately on each litter-mate (chance mutation, accidents of implantation, et cetera) are enormously more important than factors which tend to act on litter-mates simultaneously (condition of mother, et cetera).

It should be noted, however, that a minor influence from factors of the latter sort is not ruled out. Neither is it wholly hopeless to look for specific factors. A factor which showed a correlation of r with occurrence of abnormality would be responsible for a correlation of only r^2 between litter-mates. Thus one might readily detect a direct correlation of .10 or .20 between an external factor and occurrence of otocephaly and be unable to detect the resulting correlation of .01 or .04 between litter-mates.

EFFECT OF SIZE OF LITTER

The data given in table 1 can be used for one such test. Size of litter is, to a considerable extent, an indicator of the condition of the dam (WRIGHT 1922). The question whether otocephalics occur equally frequently in all sizes of litter is tested for each group and all combined in the last two columns of table 1. The total number of young in each size of litter is simply multiplied by the proportion of otocephalics in the group in question to get the expected frequency under random distribution. It will be seen that this agrees rather closely with the observed number in most cases. The grand totals, obtained as before by addition of the figures for the groups, show, however, an excess of monsters in litters of 1 and 2 and a deficiency in litters of 4 or more. The probability of obtaining such deviations by random sampling is .01 (combining the data into 3 classes: less than 3, 3, more than 3; the probability is .04 if 5 classes are retained). This is rather definite evidence that conditions indicated by a small litter have a slight excess tendency to induce otocephaly.

In the preceding paper, evidence for environmental effects was looked for in various ways. It was shown that there was no appreciable difference in frequency in first and later litters or in last litters, a factor shown later to be of considerable importance in certain other cases, including polydactyly and white spotting (WRIGHT 1926). The seasonal distribution showed however a slight predominance in winter and early spring when conditions are apt to be poor. A slightly larger percentage of the normal litter-mates of monsters died at birth and between birth and weaning than among normals in other litters from the same matings. There was a corresponding but statistically insignificant difference in birth weight between litter-mates and non-litter-mates. The monsters themselves were about 11 percent lighter than their normal litter-mates. The most significant indica-

TABLE 1

Distribution of litters of family 13 by size, number of otocephalics and family branch.

GROUP	SIZE OF LITTER	OBSERVED NUMBER OF LITTERS					EXPECTED NUMBER			TOTAL LITTERS	TOTAL YOUNG	NUMBER OF OTOS.	
		0 OTO.	1 OTO.	2 OTOS.	3 OTOS.	4 OTOS.	0 OTO.	1 OTO.	2 OR MORE			OBSERVED	EXPECTED
A	1	143	2	—	—	—	143.6	1.4		145	145	2	1.4
	2	364	8	1	—	—	365.8	7.2	0.0	373	746	10	7.2
q = .0097	3	402	9	2	0	—	401.1	11.8	0.1	413	1239	13	12.0
	4	215	8	0	0	0	214.5	8.4	0.1	223	892	8	8.7
	5	78	1	0	0	0	75.2	3.7	0.1	79	395	1	3.8
	6	15	—	0	0	0	14.2	0.8	0.0	15	90	0	0.9
Total		1217	28	3	0	0	1214.4	33.3	0.3	1248	3507	34	34.0
B	1	108	6	—	—	—	108.8	5.2	—	114	114	6	5.2
	2	189	18	2	—	—	190.3	18.3	0.4	209	418	22	19.2
q = .0459	3	185	26	0	1	—	184.2	26.5	1.3	212	636	29	29.2
	4	84	12	1	0	0	80.4	15.5	1.1	97	388	14	17.8
	5	7	2	0	0	0	7.1	1.7	0.2	9	45	2	2.1
	6	1	0	0	0	0	0.8	0.2	0.0	1	6	0	0.3
	7	0	1	0	0	0	0.7	0.3	0.0	1	7	1	0.3
Total		574	65	3	1	0	572.3	67.7	3.0	643	1614	74	74.1
C	1	12	11	—	—	—	16.6	6.4	—	23	23	11	6.4
	2	19	13	5	—	—	19.3	14.8	2.9	37	74	23	20.6
q = .2778	3	9	13	5	0	—	10.2	11.7	5.1	27	81	23	22.5
	4	7	6	1	0	0	3.8	5.9	4.3	14	56	8	15.5
Total		47	43	11	0	0	49.9	38.8	12.3	101	234	65	65.0

GROUP	SIZE OF LITTER	OBSERVED NUMBER OF LITTERS					EXPECTED NUMBERS			TOTAL LITTERS	TOTAL YOUNG	NUMBER OF OTOS.	
		0 OTO.	1 OTO.	2 OTOS.	3 OTOS.	4 OTOS.	0 OTO.	1 OTO.	2 OR MORE			OBSERVED	EXPECTED
D	1	41	0	—	—	—	39.4	1.6	—	41	41	0	1.6
	2	65	5	1	—	—	65.7	5.2	0.1	71	142	7	5.4
q=.0383	3	40	3	1	0	—	39.1	4.7	0.2	44	132	5	5.1
	4	11	3	0	0	0	12.0	1.9	0.1	14	56	3	2.1
	5	3	0	0	0	0	2.5	0.5	0.0	3	15	0	0.6
	6	1	0	0	0	0	0.8	0.2	0.0	1	6	0	0.2
Total		161	11	2	0	0	159.5	14.1	0.4	174	392	15	15.0
E	1	15	6	—	—	—	18.8	2.2		21	21	6	2.2
	2	29	6	2	—	—	29.6	7.0	0.4	37	74	10	7.9
q=.1064	3	28	7	1	0	—	25.7	9.2	1.1	36	108	9	11.5
	4	14	2	0	0	0	10.2	4.9	0.9	16	64	2	6.8
	5	1	1	1	0	0	1.7	1.0	0.3	3	15	3	1.6
Total		87	22	4	0	0	86.0	24.3	2.7	113	282	30	30.0
F	1	17	6	—	—	—	18.0	5.0		23	23	6	5.0
	2	22	9	2	—	—	20.1	11.3	1.6	33	66	13	14.5
q=.2195	3	15	12	3	1	—	14.7	12.4	3.9	31	93	21	20.4
	4	6	8	1	0	1	5.9	6.7	3.4	16	64	14	14.0
Total		60	35	6	1	1	58.7	35.4	8.9	103	246	54	53.9
Total	1	336	31	—	—	—	345.2	21.8		367	367	31	21.8
(A to F)	2	688	59	13	—	—	690.8	63.8	5.4	760	1520	85	74.8
	3	679	70	12	2	—	675.0	76.3	11.7	763	2289	100	100.7
	4	337	39	3	0	1	326.8	43.3	9.9	380	1520	49	64.9
	5	89	4	1	0	0	86.5	6.9	0.6	94	470	6	8.1
	6	17	0	0	0	0	15.8	1.2	0.0	17	102	0	1.4
	7	0	1	0	0	0	0.7	0.3	0.0	1	7	1	0.3
		2146	204	29	2	1	2140.8	213.6	27.6	2382	6275	272	272.0

tion found was in relation to size of litter, the factor considered above with larger numbers. Putting all of these indications together, there can be little doubt that unfavorable conditions tend to increase the chances of this sort of abnormal development, but that the effect is so slight that it produces no appreciable tendency for litter-mates to develop alike. In such a line as 13-13-1 (excluding 13-19-1) each otocephalus is almost wholly the result of a localized chance event, supplementing a genetic tendency common to all members of the group.

GENETIC POSSIBILITIES

Before going into more detailed analysis of the genetic situation, it will be well to review the various genetic possibilities. First is the question whether this basis is Mendelian at all or not. It may conceivably be cytoplasmic as in certain plastid characters of plants, in which case the hereditary tendency would presumably pass largely or wholly down the maternal line.

Second, even though nuclear (Mendelian) it may be the heredity of the mother and not of the young that is effective. The abundant evidence (discussed later) that monsters may be produced by toxic products has led most of those who have conceded any role whatever to heredity to suggest this mechanism (STOCKARD 1909, WERBER 1915, CHIDESTER 1923). It is well known that tendencies toward disturbed metabolism (the diabetic diathesis, gouty diathesis, et cetera) are inherited in man. WERBER has shown that butyric acid and acetone produce monsters, including cyclopia, in large numbers from treated Fundulus eggs. The argument that the frequent production of monsters by a strain may be an indication of hereditary metabolic disturbance affecting the mother is complete, except for direct evidence that it is actually true in some specific case.

A third hypothesis is that the heredity is Mendelian but is that of the egg nucleus before maturation instead of after fertilization. Experiments discussed later have shown that the most effective period for production of cyclopia in fish eggs is before first cleavage. There is no *a priori* reason then why prematuration heredity should not be the effective agent, as it has been demonstrated to be in the well known cases of voltinism and certain serosa colors of the silk worm (TOYAMA 1913), direction of coiling of the shell in Lymnaea (BOYCOTT et al 1930), larval lipochrome colors in Gammarus (SEXTON and PANTIN 1927) and various lethal effects in Drosophila (REDFIELD 1926). Since the heredity of the egg before maturation is identical with that of the mother, this hypothesis is hardly distinguishable on purely genetic grounds from the preceding. The practically random distribution within litters, discussed above, is however more easily accounted for on this hypothesis than on that of a maternal influence.

The fourth hypothesis is that the effective heredity is that of the zygote, with the sperm contributing as much as the ovum. This hypothesis may be divided according to the postulated mode of action of the gene. The time of action may be as early in development as suggested by the critical period for the production of such monsters by environmental agencies or it may be any later period. If there is early action, it means that the multiple visible effects probably trace back to a single common divergence from the normal developmental process. The alternative is separate action of the zygote heredity in the various affected tissues.

Exclusively paternal heredity (possibly of heredity transmitted by the Y chromosome) is a minor possibility as are such complications of zygote heredity as ordinary sex linkage.

The genetic basis may, of course, be a combination of two or more of these possibilities.

TESTS OF PATERNAL HEREDITY

The fourth hypothesis differs from those preceding in that the heredity of the father plays a role. It is thus of first importance to determine whether the father has in fact any influence.

It was noted in the previous paper (WRIGHT and EATON, 1923) that 711 young from matings between females of family 13 and males without ancestry in this family included only 1 otocephalus. During the period of these crosses (1916–1922), the average production of females of family 13, mated with brothers, was 2.7 percent, which yields an expectation of 19 in 711 instead of 1. This was interpreted as indicating that inheritance from the father was an immediate factor. That the mother is also important was shown by reciprocal crosses among which no otocephalus appeared among 373 young.

The above data were collected incidentally in a study of the effect of cross breeding on other characters. It has seemed desirable to make more direct tests of this important question. Accordingly females of the highest producing line (13-19-1) were mated with males from an inbred strain (family 2) which had produced no otocephalics in 3500 young up to 1925. (As noted earlier, one recent branch of this family has produced 7 otocephalics of an aberrant type.) There were no otocephalics among 88 cross-breds. The most significant results are from 5 females which were mated with brothers, either before (♀ 7979) or after the mating with the male from family 2. (Their records are given on the following page.)

The significant difference (3.7 × standard error) in the occurrence of otocephaly among the progeny of these females, depending on the breeding of the male, shows clearly that factors are introduced by the sperm which affect the fate of the developing egg. Matings between males of this line

NUMBER OF TESTED FEMALES	×BROTHER (FAMILY 13)		×♂ OF FAMILY 2	
	OTOS.	TOTAL	OTOS.	TOTAL
7979	6	19	0	2
17796	6	14	0	9
19797	4	9	0	12
19873	3	9	0	3
21171	4	15	0	5
	23	66	0	31

of family 13 with females of family 2 similarly failed to include any otocephalics (95 normal young). One male (4708) produced 7 otocephalics in a mating with a sister (31 young) but none in 14 from a female of family 2. Clearly heredity from the egg is important as well as from the sperm.

Another test of the sperm effect was made by mating two males of the high line (13-19-1, average production 27.8 percent) with females from the late generations of the large group from 13-13-1 (excluding 13-19-1) whose average production was consistently 4.6 percent. Male 24321 (from mating 13-22-20) produced 13 otocephalics among 95 young and male 24517 (from mating 13-22-2) produced 4 in 27. The 4.6 percent monsters expected from these females is raised to 14 percent and 15 percent by mating with these males from a line with a higher tendency. The difference in the case of ♂ 24321 is 4.2 times its standard error. The number from the other male is too small to be of much significance by itself but confirms as far as it goes.

These data, supplemented by that discussed in the next section in connection with sex incidence, demonstrate, it is believed, that the strong hereditary tendency toward otocephaly in the strain of guinea pigs under consideration is neither a matter of toxemia of the mothers of the monsters, nor of cytoplasmic transmission, nor of a conditioning of the egg by its nucleus before maturation and fertilization. It is, on the contrary, a function of the heredity of the egg after fertilization.

RELATION TO SEX

One specific factor of otocephaly, which is Mendelian and zygotic is easily demonstrated, namely, sex. At the time of the preceding paper, the ratio of females to males was 55 to 26 among these monsters, although there was approximate equality in the number of females and males (461 and 447 respectively) among the total progeny of the parental matings. The departure from equality among the monsters is over 3 times its standard error and was interpreted as a fairly certain indication that female sex predisposes toward development of the defect.

The data to the present time are given in table 2 in some detail and are summarized below. The usual approximate equality of number of fe-

males and males among all of the young has continued. In the last two groups, there were 52.5 percent males and 49.5 percent males respectively among the total young of determined sexes.

Group	♂	♀	?
Sporadics with no ancestry from family 13	13	21	0
Cross-breds ¼ to ¾ family 13	3	10	1
A 13-0-2 excluding 13-13-1	15	19	0
B, D 13-13-1 excluding 13-19-1 except low branches	31	57	1
C, E, F 13-19-1 and crosses, excluding low branches	45	96	8
	107	203	10

Considering the total, it appears that females have about twice as great a tendency to develop in the abnormal direction as males unless there is prenatal death and absorption of at least half of the male monsters. The difference between the sexes is unquestionably significant, the deviation from equality being about 5.5 times its standard error.

With respect to the separate groups, it is noteworthy that there is an excess of females in every case. The deviations from equality are statistically significant in the last two groups, making it certain that sex was a factor among all descendants of mating 13-13-1. Whether it was a factor from the beginning of the history of family 13 and among the sporadic cases outside of this family is less certain, although the actual excess of females makes this the presumption.

Female monsters of this general type are in excess not only in guinea pigs but in other animals. BALLANTYNE states on the authority of TIEDEMANN that both in man and in lower animals (for example, the pig) cyclopia is more common in the female than in the male. We shall return to this point later.

MENDELIAN UNITS

The analysis up to this point indicates that the differences in the otocephalic tendencies of different strains rest on a Mendelian basis. It is of interest to determine whether this consists of a large number of genes with individually small effects or a small number with large effects. The most hopeful point of attack is in the origin and history of the highest producing strain, that descended from mating 13-19-1.

It will be recalled that this high tendency appeared abruptly in the 19th generation of brother-sister mating and 6 generations after the origin of a group (13-13-1) which has given every indication of fixation of tendency in all of its subdivisions (except 13-19-1 itself) over a period of 15 years. It has been pointed out that such fixation, through attainment of homozygosis in some combination of genes, is an almost inevitable theoretical consequence of the close inbreeding practiced. Thus the very origin of the group 13-19-1 points strongly toward a mutation, the effect of which was to increase greatly the chance of abnormal development.

Having arisen, the high tendency was transmitted to at least the seventh generation in some lines but in other lines was soon wholly lost. Reference has already been made to the substrain descended from mating 13-22-5 with a record of only 2 in 121 following 3 or 4 generations of transmission of the high tendency. Two other animals from the high line when tested gave no indication of transmission of the excess tendency. One of these was female 30942 (from mating 13-24-21). Mated with a male descended from 13-22-5 she produced no monsters in 11 young. The other was a male (No. 175 from 13-23-20) which, mated with one of the daughters of the above female produced 1 otocephalus among 9 young, and mated back to two of his own daughters from this mating produced only 1 in 15. The animals of these matings were interbred or in some cases outcrossed to the large group with a record of 4.6 percent. Altogether 204 animals were produced which traced to 13-19-1 through ♀ 30942 and also through ♂ 175 (except for the 11 offspring of 30942 referred to above). These included only 10 otocephalics, or 4.9 percent, a figure which does not differ appreciably from the 4.6 percent present before the postulated mutation arose.

In marked contrast with the records of the above animals were those of four others, descended from 13-19-1. It has already been noted that ♂ 24321 (from 13-22-20) and ♂ 24517 (from 13-22-2) produced 13 otocephalics in 95, and 4 in 27 respectively. The high percentage (13.9 percent) in the first generation not only shows transmission of the high tendency but at least its partial dominance. The fact that 9 of the above monsters were males shows that the high tendency is transmissable from father to son and hence is not sex-linked (assuming males to be XY). Eight daughters backcrossed to the relatively low producing group (4.6 percent) produced 10 monsters in 82 (or 12.2 percent).

Matings were also made with two other males from the high producing line (♂ 24518 and ♂ 23652, which like ♂ 24517 came from mating 13-22-2). One of these (♂24518) had produced 4 in 15 mated with a sister, and the other, ♂ 23652, had produced 6 in 15 in such a mating (matings 13-23-23 and 13-23-20 respectively). The crossbred stock derived from the above 4 males and animals from the low producing stock, was carried for 4 generations. There were 30 otocephalics among 282 or 10.6 percent among those which traced to 13-19-1 through only one parent, while there were 54 among 246 (22.0 percent) which traced to 13-19-1 through both parents. Some matings in the last generation produced large numbers of otocephalics (for example, 7 in 12, 6 in 10, 5 in 17, 4 in 13, 4 in 6) while others of similar ancestry produced only one or none in fairly large numbers. There was continuity of high production traceable along particular lines. All of the data in short are in harmony with the hypothesis of a more or less completely dominant factor for high production which never became

fixed and was therefore lost in some lines, although retained to the end in others.

The exact extent to which this factor increased the percentage of otocephalics is an interesting question. Let Ot_2 represent this factor and its allele ot_2 be that present in the stock with an average of 4.6 percent. Males 24321 and 24517 may either have been Ot_2/Ot_2 or Ot_2/ot_2. If homozygous, all of their offspring from the outcross to the lower lines would be Ot_2/ot_2 and the percentage of monsters (13.9 percent) indicates the chance of abnormal development in a heterozygote. On the more probable supposition that males 24321 and 24517 were heterozygous, only half of their crossbred offspring would be Ot_2/ot_2 and half ot_2/ot_2. Since the incidence of defect in the latter would be 4.6 percent, that in the former must be 23.2 percent in order to give the observed 13.9 percent. The record of the next outcrossed generation suggests the heterogeneity expected with the second hypothesis but the numbers are inadequate. The total production 10 in 82 or 12.8 percent is in sufficient harmony with the expectation, 9.2 percent, of either hypothesis. The incidence in heterozygotes thus may be as low as 14 percent but is more probably in the neighborhood of 23 percent.

On any basis it is certain that dominance is not complete. The inbred high producing line produced 27.8 percent and it has been shown that recessives ot_2/ot_2 were segregating out even from the highest producing matings. Male 175, lacking the high tendency, came from a mating which produced 6 in 15 and back of this for 2 generations of brother-sister mating were records of 10 in 26, and 11 in 26. Female 30942 came from a mating which produced 1 in 3, this from one which produced 4 in 9 and back of this the same two high producing matings as above, 10 in 26 and 11 in 26. In order to account for the high record of these and other matings, it is necessary to suppose that most if not all of the homozygotes, Ot_2Ot_2, at least among the females, developed abnormally. The most probable interpretation is that matings producing between 10 percent and 20 percent abnormals were of the type $Ot_2/ot_2 \times ot_2/ot_2$ while most of those producing higher percentages were $Ot_2/ot_2 \times Ot_2/ot_2$ with perhaps a few $Ot_2/Ot_2 \times Ot_2/ot_2$.

The genetic basis of the differences among the low producing lines of family 13, and between these and other families cannot be settled conclusively. As has been noted, however, there were indications of a segregation of tendencies in the early generations, with fixation of a tendency to produce about 1.5 percent in one line from about the 13th generation (13-13-7) and of about 4.6 percent in another, also from the 13th generation (13-13-1). One might postulate a major factor for otocephaly, introduced by one of the original pair. The later history could then be accounted for as one of loss in some lines, retention and fixation in others. The differ-

ence in the percentage of incidence in 13-13-7 and 13-13-1 can be due to the fixation of different systems of minor modifiers. There is however, no such definite evidence for a major factor ot_1, as there is for the mutation Ot_2 discussed previously.

Reference has been made earlier to crosses between the high line of family 13 and a family (2) which had produced no otocephalics among 3500 young. These crosses were carried to F_3 with the results indicated below.

FEMALE×MALE	F_1		F_2		F_3	
	OTOS.	TOTAL	OTOS.	TOTAL	OTOS.	TOTAL
(13)×(2)	0	88	1	220	2	52
(2)×(13)	0	95	1	109	2	44
	0	183	2	329	4	96
	0.0 percent		0.6 percent		4.2 percent	

The appearance of otocephalics in F_2 and of as many as 4.2 percent in F_3 after disappearance in F_1 is strongly indicative of Mendelian segregation and is compatible with a single recessive major factor (ot_1) as the cause of the difference between the families. The secondary factor Ot_2 may not have been transmitted by all of the animals of family 13 used and if it were transmitted it might well produce no effect except in segregants homozygous for the primary factor.

OCCURRENCE OF HIGH GRADES

The monsters are classified by grade of abnormality, sex and strain in table 2. The majority in all strains are in grades 1 to 5 characterized by reduction in the jaws and associated parts of the skull and musculature, but with no apparent defect of the brain. Grades 6 to 12 have a similar reduction of the jaws but, in addition, defects of the brain, nose and usually eyes. In 9 cases largely from the early records, the monsters were neither preserved nor described with sufficient completeness to make the grade certain. These were probably of grades 2, 3 or 4, but may have been of grade 5 or 6. A considerable number were described sufficiently to assign to grades 3 or 4, the commonest types, but could not be allocated to one of these. The great majority have been graded from the preserved specimens.

The most interesting genetic question is the relation of the high grades with brain defect (6 to 12) to the commoner types with normal brains. In general, strains with a higher percentage of low grade monsters, also show a higher percentage of high grades. The frequency of high grades in

TABLE 2

Distribution of otocephalics by strain, grade and sex.

GRADE	NO ANCESTRY OF (13)		CROSSES INVOLVING (13)			(13) EXCL. 13-13-1		13-13-1 EXCL. 13-19-1			13-19-1 AND CROSSES LOW SEGREGATION		13-13-1 1 PARENT TRACES TO HIGH 13-19-1			13-13-1 BOTH PARENTS TRACE TO HIGH 13-19-1			13-19-1 HIGH LINE			ALL LINES			
	♂	♀	♂	♀	?	♂	♀	♂	♀	?	♂	♀	♂	♀	?	♂	♀	?	♂	♀	?	♂	♀	?	TOTAL
A				1												1				1		1	2		3
1	2					2	1	1	1		1	2	1	3			3		2	1	1	9	11	1	21
2	1	5	1	1		1	3	5	11			1	3	2		1	2		1	5		13	30		43
3		1		2	1	2	1	4	8		1	2	4	4	1	3	6		2	6		16	30	2	48
3, 4	2	4		1		3	4	2	5					1					3	7	1	10	22	1	33
4		3	1	4		3	6	8	17	1	3	2	4	6		8	21	1	8	17	3	35	76	5	116
5		1		1		1		1	2								3					2	7		9
6	1	1					1	2	1					1		1	2			1		4	7		11
7	1		1			1		1														4			4
8	1	2																		1	1	1	3	1	5
9		1				2			1			1							1			3	3		6
10	1	1					2		3		2								1			4	6		10
11																				1			1		1
12																			1			1			1
Misc.	4	2					1										2					4	5		9
A-5	5	14	2	10	1	12	15	21	44	1	5	7	12	16	1	13	35	1	16	37	5	86	178	9	273
6–12	4	5	1			3	3	3	5		2	1		1		1	2		3	3	1	17	20	1	38
Otos.	13	21	3	10	1	15	19	24	49	1	7	8	12	17	1	14	39	1	19	40	6	107	203	10	320
Young	(24,000)		(2500)			(3507)		832	744	38	195	183	137	139	6	114	126	6	111	109	14				
Percent			0.2	0.8		0.9	1.1	2.9	6.6		3.6	4.4	8.8	12.2		12.3	31.0		17.1	36.7					

	OTOS. AS PERCENTAGE OF ALL YOUNG		OTOS. OF GRADES 6–12 AS PERCENTAGE OF TOTAL OTOS.
	1–5	6–12	6–12
No ancestry of family 13	0.10	0.04	26
Crosses of family 13	0.52	0.04	7
13-0-2 (excl. 13-13-1)	0.80	0.17	18
13-13-1 (excl. 13-19-1 except low branches)	3.9	0.55	12
13-19-1 and crosses (excl. low branches)	18.1	1.44	7

13-19-1 is probably significantly greater (2.3 SE) than the frequency in 13-13-1, indicating that the mutation Ot_2, responsible for a greatly increased percentage of otocephalics, increases the frequency of high grades as well as of low grades.

One might expect, indeed, that any factor which increased the frequency of otocephalics would increase disproportionately the chance for the extreme types to appear. The opposite, however, appears to be the case. Among the sporadic cases outside of family 13, *at least* 26 percent were of high grade. At the opposite extreme is line 13-19-1 and its crosses with a total of 19.6 percent otocephalics but only 7.4 percent of the otocephalics of high grade. The difference is significant (3.2 SE). Contrasting the 7.4 percent with the 14 percent of high grades in the rest of family 13, the difference approaches significance (1.7 SE).

Females are about twice as likely to be otocephalic as males. Yet only 3 more high grade otocephalics have appeared among females than among males (20 *vs.* 17). Only one out of 10 female monsters is high grade while one out of 6 is high grade among males. The difference is not statistically significant (1.5 SE) but at least it indicates that there is no tendency toward excessive degree of abnormality among females. Thus, both in respect to strain and sex, the grade of abnormality tends to decrease as the total frequency increases.

It is interesting in this connection that sporadic otocephaly and cyclopia as described in man and lower animals is very likely to be found associated with other abnormalities, for example, in double monsters, and in single monsters with anencephaly, cephalocele, harelip, spina bifida, exomphalus, *situs inversus*, club hand or foot, polydactyly, et cetera. There has been no such tendency in family 13 apart from occasional hydrocephalus in high grades and microphthalmia in grades 5 and 6 which are probably secondary consequences. It appears that *external* factors which tend to cause otocephaly, also tend to cause other abnormalities but that the *genetic* basis in family 13 is specifically related to otocephaly.

The tendency toward specificity of type of otocephaly in particular genetic stocks is especially well illustrated by the unusual character of all of the otocephalics produced in family 2. In these 7 monsters, all called

grade 5C, there was complete absence of the inner ears although these are the most persistent organs of the head in the otocephalics of family 13 and other strains.

THE GENETICS OF ALLIED ABNORMALITIES

Where not experimentally induced, otocephaly and allied defects have usually been reported as occurring as isolated cases which have not suggested heredity as a factor. The occasional reports of two or more in a sibship have been explicable on the basis of either heredity or environmental factors according to the predilections of the author. The most closely similar abnormality to be studied genetically seems to be a "lethal head abnormality" described by LITTLE and BAGG (1924). In this the face was usually shortened, the mandible short or absent, the mouth sometimes lacking. One or both of the eyes might be rudimentary. Breeding data were interpreted as indicating a simple Mendelian recessive lethal. This is in contrast with the situation found in the guinea pigs in which more than one gene is clearly involved and each gene (including one dominant one) affects merely the *percentage* of occurrence.

It is possible, however, that the difference is more apparent than real. In LITTLE and BAGG's data there is a significant deficiency of recessives from parents known to be heterozygous. This may be due, as the authors note, either to the devouring of stillborn young by the mother or to failure of the abnormality to be manifested in all recessives. The latter hypothesis approximates to some extent that required in the guinea pig case. It may be said that the data are consistent with the hypothesis of a single main recessive semi-lethal and indeed that this hypothesis is the most probable one but it is not clear that a more complicated situation is ruled out.

WRIEDT and MOHR (1928), and MOHR (1929) have investigated a lethal abnormality in cattle (*Acroteriasis congenita*) which has points of similarity with otocephaly. The ears are small and approximated, the mandible rudimentary, the palatines lacking, the maxillae defective and the premaxillae bent downwards. These defects suggest an otocephalus of grade 5 but the regular association of these with gross defect of all of the legs (extremities lacking, anchylosis of scapula and humerus, et cetera) make comparison doubtful. They find that this trait has been appearing frequently in herds of Holstein Friesian cattle of Norway. In nearly every case it was possible to trace both parents to a particular imported bull, only a few generations back. The frequencies were in harmony with the interpretation as a simple recessive lethal and the uniformity in degree of abnormality favors this interpretation.

FELDMAN (1932) has reported briefly on a non-lethal defect of the mouse which may possibly be related to the otocephaly of guinea pigs of family

2 (grade 5C). In these mice, there is more or less reduction of the external ear. In extreme cases the middle and inner ears and other parts of the head, especially the eyes, may be affected. He states that this trait is recessive, complicated by 20 percent overlap with normal.

There are probably many more traits whose genetics is like that of the otocephalic guinea pigs than is usually realized. In the guinea pig itself, polydactyly (WRIGHT 1926, 1931) depends on a very similar situation except for the absence of lethal effects. In certain respects this is also true of white spotting (WRIGHT 1928). A well known case in Drosophila is that of reduplicated legs (HOGE 1912) in which the major gene determines merely a certain percentage of abnormals at ordinary temperatures. According to MORGAN, BRIDGES and STURTEVANT (1925), there are many such cases in Drosophila but, being inconvenient for genetic work, they are largely discarded. The outsider is thus likely to obtain an exaggerated impression of the frequency with which genes determine clear-cut, absolute effects. In birds and mammals, cases involving multiple factors and thresholds may easily simulate simple Mendelian heredity throughout experiments as extensive as it is considered practicable to make and an erroneously simple interpretation is thus likely to be assigned. In these slowly reproducing forms, the question as to what constitutes a critical demonstration of a gene is one that deserves more attention than has been paid to it.

THEORIES OF CAUSATION

In the preceding discussion we have attempted to analyze the factors involved in the occurrence of a certain category of monster in certain strains of guinea pigs. It is desirable now to take definite cognizance of the fact that we are dealing with one of the most characteristic types of abnormal development in vertebrates (and perhaps of animals in general) with regard to the causation of which there is an extensive literature. This literature is concerned almost wholly with environmental rather than hereditary factors and with ones of a much more tangible nature than the chance factors, not common to litter-mates, found to determine practically all that is not hereditary in the guinea pigs of family 13. The task is therefore not one of cataloging similar results but of attempting to bring apparently dissimilar results under a common viewpoint.

Agnathia and cyclopia, separate or combined, have doubtless been known from the earliest times as types of monsters occurring in man and the domestic animals. BALLANTYNE points out that many of the minor deities of ancient Greece had teratological characters and that the most striking element in the appearance of POLYPHEMUS may well have been suggested by a human cyclops.

We may pass rapidly over the theories of ancient times, according to

which monsters were looked upon as the result of the play of the Gods, "sports," as signs of divine power or anger or as portents. The oldest known publication on the subject seems to be a brick found in ASHURBANIPAL'S library in Nineveh which gives in cuneiform the prognostication appropriate to each of a remarkable list of monsters (BALLANTYNE, GOULD and PYLE). Somewhat allied was the belief in the influence of heavenly bodies which seemed a rational explanation of "moon calves" as long as astrology flourished. A common mediaeval belief, that monsters are of diabolic paternity was closely related to belief that they are hybrids. Finally may be mentioned the ancient and still prevalent belief that monsters are the result of "maternal impressions."

One of these theories (that of hybridism) turns out to be related to a real factor in certain cases, but there is little connection between the mediaeval superstitution and recent experimental verification with fishes.

Among ancient writers who attempted to treat the matter in a scientific spirit, one may find the germs of theories of both hereditary and environmental determination. EMPEDOCLES and DEMOCRITUS, according to BALLANTYNE, held that heredity was wholly paternal and that monsters are due to seminal aberrations, of one sort or another. ARISTOTLE extended this idea to products of both parents. There was little development of the hereditary theory of monsters until recent times. Heredity of minor defects such as harelip and polydactyly has long been recognized. Many authors of the early 19th century (for example, MECKEL) vaguely suggested germinal defect as a cause of the non-viable monsters, but heredity could hardly be taken seriously before the idea of a hereditary mechanism had reached definiteness.

The idea that abnormal development may be a result of mechanical injury and especially of pressure goes back to HIPPOCRATES and ARISTOTLE, and has continued to the present time as one requiring careful consideration in each case. DARESTE's interpretation of the monsters of birds and mammals (including cyclopia) as largely resulting from the pressure of an arrested amnion was a form of this theory which was widely accepted in the latter half of the 19th century. Here, however, pressure is not the ultimate external factor. It is generally accepted today that mechanical forces are important secondary (internal) factors in normal or disturbed morphogenesis (though not in general in the way postulated by DARESTE) but that they are of negligible importance as *ultimate* causes of monstrosity.

During the 18th century, the theory of foetal disease came into prominence under the leadership of MORGAGNI. Apart from direct destruction of tissues, disease was believed to be a factor by causing accumulation of fluid with consequent injury from internal pressure (applied to cyclopia

and otocephaly by BÉCLARD in 1816–17 and DUGÈS in 1827) or by causing amniotic adhesions to the embryo (held by E. G. ST. HILAIRE and many later authors to be the principal source of monstrosity) or by causing malnutrition or intoxication.

WILLIAM HARVEY in 1651, noted that harelip and allied abnormalities could be accounted for by arrest of development at an embryonic stage, a view extended by later embryologists such as HALLER, WOLFF and MECKEL. This theory however is again not one of *ultimate* causes. Arrested development may be the consequence of pressure, or of malnutrition, or of intoxication, or of heredity.

The frequent occurrence of abnormalities among artificially incubated chicks was doubtless recognized as far back as the practice of this art in ancient Egypt. E. G. ST. HILAIRE (in the early 19th century) seems to have been the first to make deliberate experiments. He studied the effect of shaking eggs, pricking, covering with wax or varnish, et cetera, and produced a variety of monstrous types including high grade otocephaly. Environmental disturbance was thus proved to be a real factor. These experiments were continued by I. G. ST. HILAIRE and many others. The most comprehensive series of researches was that of C. DARESTE, published in a long series of papers summarized in 1892. DARESTE found that all effective methods (shaking, varnishing the shell, abnormal temperatures) produced the same series of monsters, frequently including cyclopia and otocephaly, if applied early in incubation. The particular theory which he invoked in the case of cyclopia, arrest of the amnion, was shown to be false by RABAUD on studying sections of cyclopic chicks produced by DARESTE's method. RABAUD found that the anterior medullary plate developed abnormally from the first in cases of cyclopia.

The list of agents which could produce cyclopia and other monstrosities in the chick was greatly extended by FÉRÉ (see BALLANTYNE 1924) who not only confirmed DARESTE's results but was successful with many others, including penetrating vapors (from turpentine, ammonia, ether, chloroform, et cetera), solutions injected into the albumen of the egg (salt, glucose, many alcohols, acetone, blood, cocaine, morphine, atropine, et cetera). He found no specificity in the production of particular monsters by particular agents and (like RABAUD) no constant relation to abnormality of the amnion. He attributed the effects to malnutrition and poisoning.

While not based on experimental evidence, the conclusions reached by recent students of human monsters are of interest. BALLANTYNE (1904) after a judicial survey of previous theories, leaned toward a combination of the views of DARESTE and FÉRÉ, namely that disturbances of nutrition and toxic effects are primary and that these are especially effective in teratogeny through arrest of the amnion. With respect to cyclopia and

agnathia he noted the frequency of association with other defects as evidence that all have a common basis in pathology of the embryo.

MALL (1908, 1917) made a study of an exceptionally large number of pathological human embryos. The frequent association of monstrosity with a pathological condition of the membranes or of the embryo as a whole (*fetus compressus*, et cetera) led him to express most emphatically the conclusion that in all cases monsters are produced by external influences among which he gave first place to disturbed nutrition (usually due, he believed, to faulty implantation) and second place to injurious substances. Like many others he drew a sharp line between monsters (including cyclopia) in whose causation he believed heredity played no part whatever, and hereditary structural anomalies, such as polydactyly. WILDER (1908) drew a similar line, but because of their symmetry (Cosmobia) was inclined to put cyclopia on the genetic side.

SCHWALBE and JOSEPHY (1913) also impressed by the symmetry of cyclopia and otocephaly rejected the crude mechanical factors such as amniotic pressure. They considered the natural causes as not at all clear. While recognizing that cyclopia can be produced experimentally by external factors they held it still an open question whether an inner factor might not be the usual cause.

We turn now to experiments with lower vertebrates in which the amnion cannot be a factor.

The hypothesis that local injury might produce such monsters was supported by BORN (1897) who demonstrated that frogs with a cyclopian eye could sometimes be produced by splitting the anterior end of the medullary plate. Later LEWIS (1909) produced cyclopia and allied types in Fundulus by pricking the anterior end of the embryonic shield. Less direct in the relation between the injury and the appearance of a cyclopic eye was the result of an experiment by SPEMANN (1904) in which by ligating the egg of Triton (2-cell stage) he obtained two-headed monsters, one head of which was frequently cyclopic (occasionally both). SPEMANN interpreted the cyclopic eye as the result of fusion, following defect of the material between the eye rudiments, a conclusion with which LEWIS concurred. More recent defect and transplantation experiments by ADELMANN (1929, 1930) indicate, however, that there is equal potency with respect to eye-forming capacity in a band across the anterior medullary plate and that the explanation of cyclopia cannot be so simple as mere loss of rudiments of predetermined parts between the eyes.

With regard to agnathia, STONE (1922) has demonstrated that deficiencies in the branchial skeleton can be induced in Amblystoma larvae by removal of portions of the neural crest in medullary plate stages.

STOCKARD (1907, 1909) obtained such an extraordinarily large propor-

tion of cyclopians (50 percent in favorable cases) by treatment of Fundulus eggs (undivided or cleavage) with magnesium salts as to suggest a specific developmental effect. He suggested that cyclopia in mammals might be caused by excess of magnesium in the maternal blood. Later however (1910) he found that alcohol and other anaesthetics were as effective as magnesium salts or more so and suggested that the effect was one of this class of substances on the developing central nervous system. His theory was one of inhibition and arrest of development (of the central nervous system) instead of partial destruction followed by fusion of lateral primordia.

CHILD and MCKIE (1911) interpreted STOCKARD'S results as due to a general inhibition of developmental rate affecting most seriously the anterior medullary plate as the most active and sensitive region rather than as a region reacting to the specific substances used.

MCCLENDON (1912) showed, in fact, that a much wider range of substances than anaesthetics was effective in Fundulus (for example, KCl, NaOH, cane sugar, caffeine) but reached the conclusion that the osmotic condition was the significant one.

WERBER (1915), with the mammalian situation in mind, studied the effect of a number of products of abnormal metabolism on Fundulus eggs. He found that acetone and butyric acid produced cyclopia and many other monsters in large numbers. He held it likely that mammalian cyclopia is due to toxemia resulting from such conditions of the mother as diabetes, jaundice and nephritis and that if heredity is concerned at all, it is maternal heredity of such conditions. As to the immediate mode of action, his theory was one of destruction of sensitive primordia by the toxic agent ("differential blastolysis") and in this respect was similar to the defect theory of SPEMANN and LEWIS rather than to the inhibitory theories of STOCKARD and of CHILD.

KELLICOTT (1916) induced the usual variety of monsters, including cyclopia, merely by subjecting the eggs to low temperatures. KELLICOTT criticized the theory of disturbed nutrition of MALL, the differential blastolysis of WERBER and developmental arrest of STOCKARD as inapplicable to effects produced before cleavage or in early cleavage stages of an egg with indeterminate cleavage. He suggested instead the disarrangement of nuclear or cytoplasmic constituents or both in the egg. As regards the nucleus, this may be considered as similar to the more recent hypothesis of induced mutation or chromosome aberration. In this connection the results of BARDEEN (1907) and of BALDWIN (1919) on the effects of fertilization of toad eggs with X-rayed sperm are of interest since from present knowledge this agent seems to be the one most likely to produce effects of the kind considered by KELLICOTT. These authors obtained abnormal de-

velopment of a particular type, characterized by stunting of all parts. The eyes were especially effected but microphthalmia, not cyclopia, was produced. The picture was one of permanent injury affecting all cells and thus very different from the normal-bodied otocephalics found in guinea pigs.

Monsters of the cyclopic and more extreme types were induced in amphibia, (most successfully in frogs, *Rana fusca*) by LEPLAT (1919). He tried a variety of agents (NaCl, KCl, LiCl, $BaCl_2$, $MgCl_2$ ethyl alcohol, chloroform and chloral hydrate) at various stages of development from first cleavage to closure of the blastopore. He was most successful with LiCl, ethyl alcohol and chloral hydrate. Treatment was most effective during the period of the horseshoe-shaped blastopore. This is a decidedly later period than indicated by most other experiments.

Meanwhile, CHILD (1911) had shown that abnormalities of as similar a nature as possible to cyclopia of vertebrates could be induced in regenerating fragments of Planaria by means of agents which depress metabolism. A series of forms was produced characterized by approximation of the eyes, fusion of the eyes, absence of eyes or in extreme cases absence of the whole head. The center of defect appeared to be in the nervous system. Pieces of a given length showed higher frequencies of abnormality at successively posterior levels of a zooid, while at a given level, reduction in size of a piece increased the frequency of abnormality.

CHILD interpreted his results on the basis of his gradient hypothesis. According to this, protoplasm is a kind of material which tends to become polarized with respect to rate of metabolism in relation to the prevailing environmental stimuli, much as it is a kind of material which acquires a marked surface-interior differentiation under surface tension. While stimulation spreads from regions of high metabolism to adjacent low regions irrespective of cell boundaries, a high region tends to maintain dominance over adjacent regions approaching (but not equalling) it in level. The consequence is the development of a regular gradient even in assemblages of cells initially heterogeneous in metabolic rate. Such a pattern tends to become stabilized by material differentiation consequent on differences in metabolic rate. In the later elaboration of pattern, this material organization becomes more important than protoplasmic polarity, although the latter tendency continues to be manifested within limited regions of relatively homogeneous character. Such gradients have been demonstrated in many simple organisms including vertebrate embryos (HYMAN 1921). In general, the anterior end of the embryo develops at the high pole of the primary pattern. Experiments indicate that high levels are more immediately susceptible to injurious agents than low levels, although more capable of acclimation during, and recovery after, continued exposure to slightly depressing influences. Cyclopia and allied types are ex-

plained as the type of monster resulting from injury to the susceptible anterior pole of the primary gradient pattern.

This theory is essentially not one of the nature of the ultimate factors of abnormal development but one of the nature of protoplasm. It becomes readily explicable under it that the same type of abnormal development should result from an environmental agency or from a gene complex either of which brings about a general depression of life activities with differential effects on parts of the organism in relation to the gradient pattern.

NEWMAN in a series of papers between 1908 and 1917 showed that the same types of monsters could be produced by making wide crosses (in teleosts) as were produced by environmental agencies. He found a close correlation between rate of development and the degree of normality of the embryos. He found that the monsters which appeared could be classified in two groups, forms with inhibited apical parts (cyclopia, microphthalmia, defective heart) and forms with inhibited basal parts (relatively large head, stunted trunk and tail, in extreme cases isolated eyes or hearts, explicable as due to differential acclimation or recovery.

STOCKARD (1921) confirmed the production of cyclopia, as well as of other types of monsters including twins, by low temperatures. His general conclusions in this paper are in essential agreement with those of CHILD, but emphasize especially the significance of critical moment. After noting (1) that all types of monsters, double and single, may be caused by the *same treatment* and (2) that one type (as cyclopia) may be produced by a great number of different treatments and (3) that all effective treatments are ones which tend to lower developmental rate he concludes (4) that the type of monster depends on the particular developmental moment or moments during which developmental rate is reduced. Thus he finds that the critical moment for twinning comes first followed shortly by those for cyclopia, brain vesicles, the branchial arches and the inner ear.

These principles are in accord with CHILD'S theory except for some qualification of the fourth. Different types of abnormality are to be expected on any basis from treatment after the organs concerned have been formed, as compared with treatment before; and apart from this, the regions of most active metabolism at one stage are not necessarily the same as those of another stage. Under CHILD'S theory, however, radically different types of monsters (due respectively to differential inhibition and differential acclimation) are to be expected from treatment at the same developmental moment, depending on the severity of the treatment or the physiological state of the animals.

On reviewing the accounts of the various experiments on production of monsters, one gets the impression that the critical moments are not as definite as implied by the above theories. Results obtained by HINRICHS

(1925) on treating Fundulus eggs with ultraviolet are especially significant since only a few minutes of treatment were necessary but in general they agree with those of earlier workers, including STOCKARD, as far as comparison is possible. The earlier the treatment the greater the number of monsters. Thus 3 minutes of treatment produced 100 percent monsters if within 10 minutes after fertilization, but only 25 percent at 75 minutes, and only about 5 percent at 3 1/2 hours or at 21 hours (when the axis was in process of formation). The monsters induced by treatment in the one cell stage (first 2 1/2 hours) included a great variety of types: twins and anterior and posterior duplicities, defective hearts, cyclopia, defects of branchial arches (otocephaly), dwarfs, forms with stunted tails, et cetera. Twins and anterior duplicities could not be produced by later treatment but all of the other types could be produced by treatment during cleavage although with decreasing frequency. With treatment after the axis had begun to form, eye defects took the form of microphthalmia rather than cyclopia.

It is to be noted that the effective period for cyclopia is before the appearance of morphological rudiments of eyes, whether the agent be ultraviolet, chemicals or low temperature. It apparently extends, however, from the unsegmented egg to a period shortly after formation of the medullary plate.

These results point to a similarly early time of action of the genes in the guinea pigs discussed here rather than to separate action on the primordia of the various organs.

The results in the inbred line of guinea pigs are, however, in marked contrast to those of experimental treatments in the uniformity of monstrosity produced (apart from mere variation in degree). This probably means that the gene effects are relatively direct and precise in their moments of action, while the response to the experimental agents varies greatly in its promptness, dependent on the physiological state of the organism.

It is interesting in this connection, to return to consideration of the excess of females among otocephalics. As already noted, a similar excess has been noted among cyclopians of man and other animals. It is stated (HIRST and PIERSOL, SCHWALBE) that human double monsters (Dicephalus, Pygopagus) are two or three times as likely to be female as male. In both natural and experimentally produced two-headed monsters, one head is likely to be cyclopian or otocephalic. It may be noted here that two double monsters have occurred in our stocks of guinea pigs. Both were of the cruciate type, two bodies, belly to belly, united anteriorly; in one case with two heads, the other with one head (but four ears) at right angles to the body axes. In the two-headed case one face was normal, the other

otocephalic of grade 6 (Syncephalus thoracopagus janiceps). The tendency toward association of otocephaly, cyclopia and duplicity point toward a close relationship in causation. As noted above, all of these types are produced only by early experimental treatment. The excess of females in all of them suggest that the X chromosome is effective as a primary sex determining factor perhaps through an effect on rate of metabolism near the beginning of development, although differential absorption at a later period is a possible alternative.

CONCLUSIONS AND SUMMARY

Otocephaly is a well known type of monstrosity in vertebrates. In low grades, the defect is restricted to the jaws (agnathia). In higher grades, extreme jaw defect is associated with defect of the brain and associated parts, cyclopia being a common manifestation. In the highest grades, jaws, nose, eyes and all of the brain in front of the medulla are lacking (aprosopus).

Otocephaly is very rare in most strains of guinea pigs (less than 0.05 percent). A particular inbred strain produced 1 percent in its early generations. A branch which arose in the 13th generation of brother-sister mating has produced about 5 percent in all but one of its sub-branches for 15 years. The exceptional branch has produced about 27 percent but tends continually to relapse to 5 percent.

The usual theory has been that this sort of monster is produced in mammals by maternal toxemia and that any tendency to heredity is maternal heredity. Experiments show that this is not the case here. The pertinent heredity is that of the monster itself, equally derived from both parents.

There is evidence of segregation of different tendencies among branches of the family in the early generations. The 5 percent branch must be considered as homozygous for a certain gene complex. It is shown that within this branch the monsters cannot be due to ordinary Mendelian segregation and that there are no balanced lethal factors. Neither can the monsters be due to any important extent to environmental factors of such a sort as to act on litter-mates alike. The randomness of occurrence within litters of each size, indicates that each monster is due to a highly localized chance event, supplementing a genetic tendency common to all members of the group. There is, however, a slight excess in small litters, indicative of some effect of unfavorable conditions.

The evidence indicates that the abrupt change from 5 percent to 27 percent occurrence in one sub-branch in the 19th generation was due to a dominant mutation. This has segregated as a unit in later generations and in outcrosses to the 5 percent branch.

Sex is a factor, there being twice as many females as males among the monsters.

There is evidence of genetic differences in the type of defect. Only 7 percent of the otocephalics of the high producing line show brain and eye defect, while over 26 percent of the sporadic otocephalics of other families were of these extreme types. All 7 of the otocephalics of one inbred strain showed an earless condition not found in any other strain.

It is pointed out that these monsters are similar to ones produced by a great variety of environmental agents (mechanical, chemical, ultra-violet, cold, et cetera) in lower vertebrates. More or less closely allied types have been produced by treatment as early as the uncleaved egg but apparently cannot be produced by treatment later than an early medullary plate stage. It is suggested that the genetic factors in this case act by bringing about (directly or indirectly) a general depression in vital activity at a particular critical moment, with permanent effects on the development at the anterior end of the embryonic axis as the most active and hence most susceptible region at the time of action.

It is noted that in the case of the guinea pigs studied the genetic factors play a less decisive role with respect to the fate of individuals than reported for several other somewhat similar types of abnormality. The genetic complex determines merely the percentage incidence in a strain, while chance differences of such a nature as to be practically as great between litter-mates, as between non-litter-mates of the same genetic constitution, are decisive with respect to individuals.

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AN ANALYSIS OF VARIABILITY IN NUMBER OF DIGITS IN AN INBRED STRAIN OF GUINEA PIGS*

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Received December 14, 1933

THE DIGITS OF GUINEA PIGS

Guinea pigs normally have four digits on the front feet (no thumb) and three digits on the hind feet (digits I and V absent). This condition is found in all wild species of the family Caviidae and in the closely allied family Hydrochoeridae.

A larger number of digits is found in general in the species of other families of the hystricoid rodents. The reduction in the Caviidae and Hydrochoeridae is thus in all probability a very ancient character.

It is a frequently stated principle of paleontology (DOLLO's law) that lost parts never return. Nevertheless, it is well known that an extra digit, resembling in every respect (when well-developed) a normal little toe is not an especially uncommon occurrence in the guinea pig. CASTLE (1906) found a guinea pig with one imperfectly developed little toe and was able by selection and inbreeding to build up a race in which this digit was invariably present and perfectly developed including bones, muscles and nail and even a new plantar tubercle on the foot. I have found a similar little toe in several independent stocks. It has also been described by STOCKARD (1930) and by PICTET (1932). The appearance of the little toe in two grades of development is shown in figure 1. Its position and structure have been the same in all stocks in which it has been observed. This type of polydactyly must be sharply distinguished from the duplication of a digit which has occurred associated with other abnormalities of the foot (figure 2) in a few cases in stocks which have no special tendency toward the development of the reversionary type.

A series of grades of development of the little toes has been used in the records but for the purposes of this paper two will suffice: "Good" in which both little toes are full-sized and so firm that they do not bend back laterally to the foot on moderate pressure, and "poor" including all lower grades. Grades of perfection are recognizable within the category "good" but the above line of cleavage seems to be the most objective one which can be made.

* Part of the cost of the accompanying tables is paid by the GALTON AND MENDEL MEMORIAL FUND.

PREVIOUS GENETIC RESULTS

CASTLE (1906) found that the extra-toed condition was transmissible both by males and females and that the degree of transmission was closely related to the grade of development. His original male with only a weak fourth toe on one foot had 25 percent (9 out of 36) extra-toed young from

FIGURE 1.—Sketches of left hind feet of guinea pigs. The normal 3-toed condition is shown at the left. At the right is a foot with well-developed little toe and a corresponding plantar tubercle. Between is a grade of imperfect development of the little toe.

related females and 6 percent (2 out of 32) from unrelated ones. Four out of 9 young from polydactylous females were polydactylous. In the next 3 generations selected males produced increasing percentages of polydactyls from the various classes of females but even matings between perfect

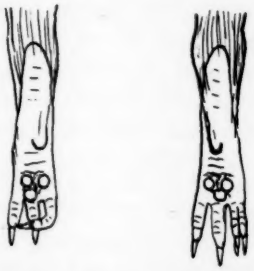

FIGURE 2.—Hind feet of an abnormal guinea pig, showing imperfect development of digits on left foot and duplication of a digit on the right foot. This type of polydactyly is to be distinguished from the atavistic type of figure 1.

polydactyls produced some normals as well as many with imperfect fourth toes. A close approach to fixation of the perfect fourth toe was reached in generation 5. Complete fixation was later attained and has continued to the present in a branch of this strain in my possession. CASTLE noted a slight excess tendency toward extra toes on the left side (630 left, 589 right).

The breeding results could be accounted for neither on the basis of a simple recessive, nor of a simple dominant. CASTLE concluded that the extra toe was probably inherited in a manner intermediate between blending and alternative inheritance and that there was some sort of latency in normal guinea pigs. In a later publication (1911) he noted that "an alternative explanation is possible that the development of the fourth toe depends upon the inheritance of several independent factors and that the more of these are present the better will the structure be developed."

STOCKARD and PAPANICOLAU (1920) concluded that the character is a Mendelian dominant. In a more recent paper (1930) however, STOCKARD reaches the conclusion that the mode of inheritance must be more complex. He has found it occurring occasionally in eight distinct stocks and considers it probable that it is latent in all stocks. The results of matings were in the main similar to those reported by CASTLE. Normal×normal produced 11 percent (22 out of 199) with the extra toe. Conversely when both parents were four-toed, 25 percent (53 out of 214) of the progeny were normal. Contrary to CASTLE he obtained no normals among 35 young, both of whose parents had perfect fourth toes. In all cases in which both parents were of the same grade, this grade was in excess among the progeny. The proportion of four-toed young from matings in which one parent was four-toed and the other normal varied with the grade of the former and the source of the latter.

PICTET (1932) has recently reported on experiments with this character. Extra-toed animals appeared in a certain stock which had previously produced only normals, 3000 in number. The 7 pairs which produced polydactyls at all, gave a total of 9 polydactyls in 144 young. The following table gives in condensed form the results obtained in eight later generations in which both parents trace in part at least to this foundation stock.

	TOTAL	4-TOE	PERCENT 4-TOE
Normal × Normal	196	9	4.6
Normal × 4-toe	148	47	31.8
4-toe × 4-toe	173	121	69.9

As in CASTLE's and STOCKARD's data, matings of polydactyls with unrelated normals gave smaller percentages of polydactyls (15 in 101 or 14.9 percent) than matings with related normals. PICTET's results resembled CASTLE's in the excess of left little toes over right ones (46 left only, 18 right only, 139 both). He could find no heredity of the asymmetry.

PICTET attempted to reach a definite factorial analysis. His reasoning may be summarized as follows: normal×normal may give ratios approxi-

mating 15 normal to 1 polydactyl. This requires a dihybrid ratio at least. But polydactyly cannot be due merely to a combination of two recessives implied by the 15:1 ratio, since polydactyl×polydactyl may produce normals. Thus a third gene is required. The specific hypothesis put forward is that polydactyly depends on the combination of *PP* with at least two positive genes from the series *E*, *e* and *N*, *n*. The six combinations *PPEENN*, *PPEENn*, *PPEEnn*, *PPEeNN*, *PPeeNN*, and *PPEeNn* are 4-toed while the other 21 combinations are normal.

He assumes that all of the original matings of normal×normal which gave polydactyly were of the type *PpEenn*×*PpeeNn* yielding 15 normal to 1 polydactyl as observed. Matings between polydactyls from these (*PPEeNn*) would then be expected to yield 5 normals to 11 polydactyls. The observed F_1 ratio, 9 normals to 19 polydactyls, is in agreement. The ratio of 43 normals to 102 polydactyls from all matings between polydactyls from the next 7 generations is pointed to as additional evidence, overlooking that most of the polydactyls of these generations would be homozygous in *E* or *N* or both and would give higher percentages.

In the cases of normal×normal and normal×polydactyl, he finds it possible to yick out formulae for the parents which will account for each type of ratio observed. It must be taken into account, however, that with 6 different genetic constitutions assigned polydactyls and 21 to normals there are 126 possible types of matings of normal with polydactyl and 210 between normals. This gives considerable room for choice of formulae to fit observed cases. Under such conditions the demonstration that a choice of formula is capable of accounting for observed ratios does not constitute proof that the formulae are correct.

The whole argument also ignores the fact already demonstrated (WRIGHT 1926) that non-genetic factors play such an important role that normals and polydactyls may be of the same genetic constitution. It may be concluded that the specific genes *P*, *p*, *E*, *e* and *N*, *n* cannot be taken seriously.

My own experiments began in a preliminary way in Dr. CASTLE's laboratory in 1914. The results showed clearly that further advance was hardly possible without the use of stocks known to be homozygous or nearly so, that is to say, of closely inbred stocks. Shortly thereafter I had an opportunity to study the records of such stocks. A considerable number of inbred lines were started at the experiment station of the U. S. Bureau of Animal Industry at Beltsville, Maryland in 1906 and maintained by brother-sister mating. Among the 23 which persisted more than a generation or two, it was found (WRIGHT 1922) that 12 had produced no polydactyls in a total of 8400 young (1906 to 1915) as shown by the records (which however merely noted the occurrence of polydactyly, not of normality during this period). Five of the strains had produced a total of 12

polydactyls in 4600 young or 0.26 percent. The remaining 6 strains had produced polydactyls in percentages ranging from 1.7 percent to 19.0 percent.

TABLE 1

Occurrence of polydactyls in 23 inbred strains.

STRAIN	4-TOED	TOTAL	PERCENT 4-TOED
1, 3, 9, 13, 15, 18, 19, 20, 21, 23, 32, 34	0	8404	0
2, 7, 14, 17, 39	12	4626	0.3
24	19	1142	1.7
36	26	1298	2.0
11	25	1151	2.2
38	59	768	7.7
35	181	1343	13.5
31	152	802	19.0

A study of the histories of these families, especially of 35 and 31, led to the conclusion that "the segregation among the family lines is so sharp that it is probable that a careful investigation of polydactyly would yield Mendelian results, though much nongenetic variation must be present."

Since 1916 the number of digits on the hind foot and the grade of extra toe if present was made a matter of routine recording for every animal born in the colony of the Bureau of Animal Industry. The positive notation of normality doubtless increased the reliability. A large branch of family 35, descended from a single mating in the 12th generation of brother-sister mating, was made the object of intensive study with regard to non-genetic factors. Important effects of age of parents (presumably of dam) and of season of birth were demonstrated and it was shown that these constituted only a part of the total non-genetic variability (WRIGHT 1926). In the same paper the results of crosses between certain of the strains (2, 13, 32, 35) and Professor CASTLE's strain of perfect polydactyls [*D*] were reported briefly as indicating "segregation of two or three major genetic factors, the results being clearly different in crosses with different 3-toed stocks."

The present paper will present more fully than before and on the basis of additional data, the analysis of the variability within the inbred strain No. 35. It is hoped to present in detail in later papers the results of the crosses between inbred strains referred to above, the results of linkage tests and the results in a strain (I) in which pollex and hallux appear as well as little toe.

DIFFERENTIATION OF SUBSTRAINS OF FAMILY 35

The branching lines of descent from the foundation mating of family 35 are shown in figure 3 and table 1. Four branches (B, C, E and G) started

in the second generation. Three of these produced low percentages of 4-toed young, while one (C, D) produced none at all among 335 young in a history extending through the 12th generation. One of the other branches of the 2nd generation (E) split off a branch (F) in the third generation which never produced polydactyls. These early differences may easily have been the result of segregation of factors heterozygous in the original pair.

Of greater interest are the records of the later branches, all descended from a single mating in the 12th generation of brother-sister mating. Under such mating, some combination of genes should have become nearly fixed

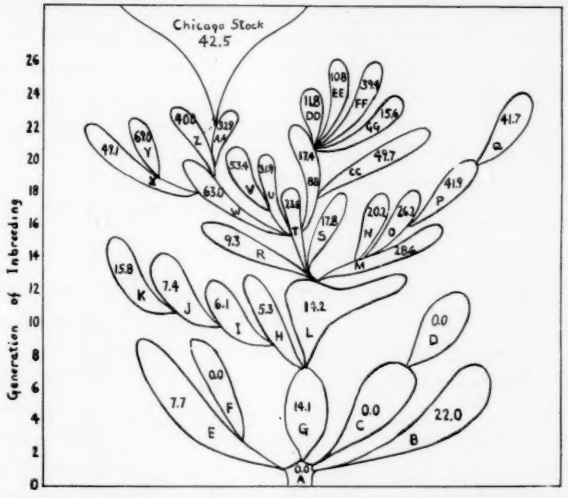

FIGURE 3.—Branches of family 35 showing percentages of polydactyly.

by the 12th generation, assuming of course the absence of new mutations or selection for heterozygosis. The theoretical rate of decrease of heterozygosis is 19.1 percent per generation, under which, 92 percent of the genes, not similarly homozygous in the foundation pair of guinea pigs, should have become homozygous. The percentage of unfixed genes should be approximately halved with each additional 3 generations of brother-sister ancestry.

All of the 21 substrains, descended from the single mating in the 12th generation, indeed nearly all of the matings, have produced polydactyls. The mere occurrence of both 3-toed and 4-toed young would indicate continued heterozygosis on such an interpretation as that of PICTET. It does not necessarily do so, however, if non-genetic factors play a role. The difference in percentage among the strains (9 percent to 69 percent) are, however, indicative of real genetic differentiation.

TABLE 2

Percentage of polydactyls in different branches of family 35.

BRANCHES			MATINGS	YOUNG	
SYMBOL	FIRST MATING	PARENT BRANCH	NUMBER	NUMBER	PERCENT 4-T
A	0–1	—	2	18	0.0
B	2–3	A	13	109	22.0
C	2–2	A	19	152	0.0
D	8–6	C	17	183	0.0
E	2–4	A	13	117	7.7
F	3–12	E	7	88	0.0
G	2–1	A	11	99	14.1
H	8–1	G	12	152	5.3
I	9–1	H	8	98	6.1
J	10–2	I	18	215	7.4
K	11–13	J	9	76	15.8
L	8–2	G	14	148	14.2
M	13–3	L	5	49	28.6
N	14–3	M	6	104	20.2
O	14–7	M	9	122	26.2
P	16–3	O	10	124	41.9
Q	20–14	P	7	60	41.7
R	13–7	L	5	43	9.3
S	14–12	R	14	157	17.8
T	14–5	R	10	123	23.6
U	16–4	T	6	69	31.9
V	17–7	U	5	58	53.4
W	16–7	T	5	54	63.0
X	18–2	W	5	53	49.1
Y	19–5	X	11	84	69.0
Z	19–3	W	9	100	40.0
AA	20–17	Z	10	79	32.9
BB	16–9	T	10	121	17.4
CC	18–7	BB	16	167	49.7
DD	21–3	BB	15	136	11.8
EE	21–4	BB	12	130	10.8
FF	21–8	BB	8	66	39.4
GG	21–18	BB	9	77	15.6

Comparison of the observed frequencies of 3-toed and 4-toed young in the 21 substrains with those calculated from the percentages in the grand total (31.07 percent 4-toed) yields a X^2 of 247. The probability that such a value of X^2 could arise by random sampling (20 degrees of freedom) is indefinitely small. There may, however, be some other factor than heredity tending to cause correlated occurrence within a substrain. It is shown later that there is an important correlation between litter-mates which is not

genetic in origin. But even if litter-mates were invariably identical with respect to number of digits, so that number of litters instead of number of individuals should be used in calculating X^2, the value of the latter would still be as great as 101 (247 divided by 2.44 the mean size of litter) and this still has no appreciable chance of arising by random sampling. There seems to be no other factor which brings about correlated occurrence to an important extent and as X^2 could be reduced to 50 and have a chance of only about .0002 of origin by random sampling, there can be no doubt of the reality of the genetic differentiation among the substrains.

On comparing each substrain with that from which it was derived, it will be seen that there has been some tendency toward persistence. The correlation between successive substrains is +.47. There are at least half a dozen cases, however, of changes of percentages which appear important. Their interpretation as due to segregation is rather unlikely, in view of the many generations of brother-sister mating back of some of the most striking ones. The most plausible explanation seems to be the occasional occurrence of minor mutations followed by segregation.

DEGREE OF DETERMINATION BY HEREDITY

It is of interest to determine the portion of the total variability due to substrain differentiation. But at once we encounter the difficulty that the character does not occur in grades from which the variance can be calculated directly. The most significant classification is merely the dichotomy 3-toed or 4-toed.

It is already clear that this dichotomy cannot correspond to alternative phases of a single factor. It is the result of a physiological threshold in a character affected by many factors. It is therefore reasonable to assume that there is a scale of factor combinations to which each factor makes a fairly constant contribution and that variabilities may be compared on such a scale. As the unit of measurement it is convenient to take the standard deviation within a substrain ($\sigma_s = 1$). The deviation of the mean (m) of each substrain from the threshold for polydactyly can be found from a table of probability integrals on the assumption that the distribution within each group is normal on such a scale as described. Thus for the deviation of the mean of the group, $m = \text{prf}^{-1}(q - \frac{1}{2})$ where q is the proportion above the threshold and prf^{-1} is the inverse probability integral, where $\text{prf } x = \int_0^x \frac{e^{-z^2/2}dz}{\sqrt{2\pi}}$. The variance of such means can now be found by the usual formula

$$\sigma_m^2 = \frac{\Sigma(m - \overline{m})^2 f}{n} = \frac{\Sigma m^2 f}{n} - \overline{m}^2$$

where $\bar{m}$ is the weighted average of the means, $\left(\bar{m}=\frac{\Sigma mf}{n}\right)$. If the set of substrains are thought of as a random sample from a large number, correction should be made for the uncertainty of the grand average ($\bar{m}$) by multiplying by $\frac{h}{h-1}$ where h is the number of substrains (assumed to be of approximately the same size). If, on the other hand, the groups be thought of, not as a sample, but as including *all* subdivisions of the population, no such correction should be made.

In the present case the question at issue is the proportion of the variance of the actual population due to the differentiation of its substrains, rather than proportion in a hypothetical population made up of an indefinite number of such substrains. This correction is accordingly not made in the final estimate. It may be noted that it is even less justified in cases analyzed later, such as subdivision by age of mother and month of birth.

Another correction is for the variance of means due merely to accidents of sampling within the substrains. The formula for the variance of means of groups consisting of f individuals is $\frac{1}{f}\left(=\frac{\sigma_s^2}{f}\right)$. The value $\frac{h}{n}$ may be taken, if the groups are approximately the same size. In the present case this correction is not very important $\left(\frac{h}{n}=.0106\right)$.

The variance of the total population is the sum of the variances within groups and of group means:

$$\sigma_T^2=\sigma_s^2+\sigma_m^2=1+\sigma_m^2.$$

The proportion of the total variance due to substrain differentiation is thus $\frac{\sigma_m^2}{1+\sigma_m^2}$. This does not differ appreciably, in most cases, from PEARSON's formula for squared biserial eta, but does not involve the assumption that the total distribution is normal.

For the 21 substrains of table 3 the above formula gives 19.2 percent if no corrections are made, 18.5 percent if correction is made for average size of subgroup and 19.3 percent if both corrections are made. The second of these may be taken as the best estimate of the proportion of the variance of the actual population due to differentiation of its substrains.

This differentiation of substrains with respect to polydactyly contrasts with the relative uniformity of the same substrains in most other respects

(WRIGHT and EATON 1929). The family is characterized for example by a tricolor pattern. The percentage of white varies in individuals from 5 percent to 100 percent but the substrains derived from the same mating in the 12th generation vary only from 60 to 73 percent and wholly at random. The parent offspring correlation of only $+.024 \pm .013$ with respect to amount of white confirms the view that in this respect no appreciable portion of the variability since the 12th generation is genetic.

THE CHICAGO STOCK

The data above refer to the history of family 35 from a mating in the 12th generation from 1916 to 1924 inclusive. A small stock has been maintained in Chicago from 1926 to the present. It has not been practicable to continue the system of mating brother with sister but the entire stock traces to a single Beltsville mating in the 22nd generation of inbreeding. This Chicago branch has produced 152 polydactyls among 358 young through June 1933, an average of 42.5 percent. This does not differ very much from the record of 32.9 percent produced by the Beltsville substrain (AA) from which it came and still less from the next most closely allied Beltsville substrain (Z with a record of 40.0 percent).

It may be noted that the Beltsville records were made wholly as a matter of routine, no greater attention being paid to strain 35 than to any other. No statistical analysis was started until after the records through 1924 were complete. The Chicago data on the other hand were recorded with especial care after such an analysis had been made on the Beltsville data.

CORRELATION BETWEEN PARENT AND OFFSPRING

In the Beltsville stock (table 3, next to last column) 3-toe×3-toe produced 26.6 percent polydactyls, while 4-toe×4-toe produced 41.6 percent. This does not indicate much genetic difference between normals and polydactyls within the stock, perhaps no more than expected on the basis of the substrain differences demonstrated above.

To test this matter, the results of matings should be compared *within* substrains or within groups of similar substrains. The data are divided into four such groups (I with 9–16 percent, II with 17–29 percent, III with 32–42 percent and IV with 49–69 percent polydactyls respectively) in table 3. The last column gives the average percentage from each type of mating on weighting the percentage within each group from that type of mating by the total number of young from that group.

It appears that *within* groups, 4-toe×4-toe produced on the average even fewer polydactyls than did 3-toe×3-toe (27.2 percent compared with 30.6 percent) but the difference has no significance. The result from

TABLE 3

Results of different types of matings within 4 subdivisions of family 35. Number of matings (Mat.), number of young (Y) and percentage of polydactyls (percent 4-T) are recorded.

DAM	SIRE	GROUP I (9 PERCENT–16 PERCENT)			GROUP II (17 PERCENT–29 PERCENT)			GROUP III (32 PERCENT–42 PERCENT)			GROUP IV (49 PERCENT–69 PERCENT)			TOTAL			
		MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	WEIGHTED 4-T PERCENT
3-toe	3-toe	31	269	14.9	35	414	20.3	23	204	36.3	12	132	55.3	101	1019	26.6	30.6
3-toe	4-toe	4	31	9.7	8	74	33.8	6	49	49.0	5	31	71.0	23	185	40.0	40.8
4-toe	3-toe	6	79	3.8	8	114	20.2	3	35	40.0	2	42	47.6	19	270	22.2	27.8
4-toe	4-toe	2	7	0.0	5	74	17.6	17	210	37.6	23	211	55.5	47	502	41.6	27.2
	Total	43	386	11.9	56	676	21.5	49	498	38.4	42	416	55.8	190	1976	31.1	

3-toe×4-toe diverges considerably from the others but depends on too few matings to be given much importance. It is clear that the factors responsible for differences in the number of toes among animals of the same substrain must be practically wholly non-genetic.

Returning to the total population, it is of interest to find the correlation between parent and offspring. Tetrachoric coefficients were obtained by PEARSON's formula from the totals of table 3, based on 1976 offspring but only 190 matings. (Most of the tetrachoric correlations given in this paper were calculated from very convenient graphs prepared by my colleagues Prof. L. L. THURSTONE and LEONE CHESTRE in mimeographed form.) The assumption is again made that there is a graded scale of factor combinations back of the alternative categories, normal and polydactyl. Similar correlations were obtained from each of the four subdivisions of the data and averaged.

CORRELATION	TOTAL POPULATION	AVERAGE OF 4 SUBDIVISIONS
Offspring with dam	+.106	−.102
Offspring with sire	+.261	+.086
Average	+.183	−.008

It is again brought out that there is no genetic variability *within* the substrains. Even in the total population environmental differences are clearly much more important than genetic ones.

With homozygosis within strains, a correlation of +.183 between parent and offspring indicates that 18.3 percent of the variance of the total stock (on the scale of factor combinations) is due to hereditary differences and 81.7 percent to environmental ones. This agrees well with the estimate of 18.5 percent of the variance as due to substrain differentiation previously reached.

The results in the Chicago stock, not included in the above, are given in table 4 below.

TABLE 4

Results from different types of matings in Chicago stock.

DAM	SIRE	MATINGS	YOUNG	PERCENT 4-TOE
3-toe	3-toe	24	140	41.4
3-toe	4-toe	13	71	29.6
4-toe	3-toe	11	70	41.4
4-toe	4-toe	16	77	57.1
		64	358	42.5

There is a slight indication of hereditary difference between 3-toed and 4-toed animals but the number of matings is too small to attribute much significance to it. It is interesting that the tetrachoric correlation between dam and offspring (+.19) is here greater than that between sire and offspring (+.04) which is the opposite of the situation in the Beltsville stock. The average parent-offspring correlation (+.11) is not certainly significant even on the basis of a standard error calculated from number of offspring. Taken at face value, however, it would indicate about the same proportion of the variance to be genetically determined as in the Beltsville data since under *random* mating a correlation of +.11 between parent and offspring implies 22 percent determination by heredity. We shall return to this question later.

CORRELATION BETWEEN LITTER-MATES

Most tangible environmental factors should act alike on litter-mates. If such factors are important there should be a tendency for litters to consist wholly of 3-toed or wholly of 4-toed young. Table 5 shows an analysis

TABLE 5

The distribution of polydactyls in litters of 2 or more in the Beltsville and Chicago stocks. The theoretical values (Calc.) are derived from binomial expansions based on the percentage of polydactyls in each group.

SIZE OF LITTER		NUMBER OF 4-TOE IN LITTER						NUMBER OF LITTERS	NUMBER OF YOUNG	PERCENT OF 4-T
		0	1	2	3	4	5			
Beltsville 2	Obs.	146	76	64	—	—	—	286	572	35.7
	Calc.	118.4	131.2	36.4	—	—	—			
	Diff.	+27.6	−55.2	+27.6	—	—	—			
Beltsville 3	Obs.	119	63	34	46	—	—	262	786	34.2
	Calc.	74.6	116.4	60.5	10.5	—	—			
	Diff.	+44.4	−53.4	−26.5	+35.5	—	—			
Beltsville 4	Obs.	44	23	11	10	5		93	372	25.5
	Calc.	28.6	39.2	20.2	4.6	0.4				
	Diff.	+15.4	−16.2	−9.2	+5.4	+4.6				
Beltsville 5	Obs.	9	6	0	1	0		16	80	11.3
Beltsville 6	Obs.	1	0	0	1	0		2	12	25.0
Chicago 2	Obs.	26	14	9				49	98	32.7
	Calc.	22.2	21.6	5.1						
	Diff.	+3.8	−7.6	+3.9						
Chicago 3	Obs.	16	11	12	13			52	156	47.1
	Calc.	7.6	20.4	18.4	5.6					
	Diff.	+8.4	−9.4	−6.4	+7.4					
Chicago 4	Obs.	3	4	4	3	1		15	60	35.4
Chicago 5	Obs.	0	1	0	0	1	1	3	15	66.7

of litters of 2, 3 and 4 in the Beltsville stock from this standpoint. The litter sizes are those after deducting unclassified young (feet eaten when found). The observed frequencies (Obs.) of litters containing no polydactyls, one, two, et cetera are compared with frequencies (Calc.) expected on the assumption of random occurrence in litters. Litters of 2 contained 35.7 percent polydactyls. If there were no tendency to concurrence in litters, the chance of occurrence of 0, 1 and 2 polydactyls (D) respectively should be according to the terms of the expression $(.643\ N+.357\ D)^2$. Similarly in litters of 3, there were 34.2 percent polydactyls and the frequency of different sorts of litter should be in accordance with the terms of $(.658\ N+.342\ D)^3$. In litters of 4 there were 25.5 percent polydactyls and we expand $(.745\ N+.255\ D)^4$ to find the expected proportions.

It will be seen that there was a marked excess of litters wholly normal or wholly polydactyl with corresponding defect of mixed litters (except in the case of 3 polydactyls in litters of 4).

A similar analysis is presented of the less numerous Chicago data for litters of 2 and 3. Here again there is an excessive number of litters which consist wholly of one or the other sort of young and a deficiency of mixed litters. The χ^2 method can be used to test the significance of these differences. Two degrees of freedom are lost in each case through acceptance of the total numbers and the total percentages of polydactyls in each size of litter. Because of small numbers, litters with 3 and with 4 polydactyls are combined in the case of litters of 4 in the Beltsville data.

	LITTER SIZE	χ^2	n	PROB.
Beltsville	2	50.6	1	.000,000
	3	182.5	2	.000,000
	4	39.2	2	.000,000
Chicago	2	6.3	1	.012
	3	25.6	2	.000,000

There is obviously no doubt of the reality of the tendency to concurrence of polydactyly in litter-mates. This contrasts with the low fraternal correlation with respect to white spotting in the same material and the absence of any tendency toward concurrence of otocephaly in another inbred family.

It is interesting to throw this result into the form of the correlation between litter-mates. This can be done by making up 2×2 tables from the data and calculating the tetrachoric coefficients of correlation. Standard errors have been calculated by PEARSON'S formula quoted by KELLEY (1923, p. 258) multiplied by a term to allow for the repetition of indi-

viduals in litters larger than 2, namely, $\sqrt{\frac{1+(L-1)r}{1+r}}$ where L is size of litter.

BELTSVILLE DATA			CHICAGO DATA		
SIZE OF LITTER	r_{oo}	σ_r	SIZE OF LITTER	r_{oo}	σ_r
2	.62	±.07	2	.53	±.19
3	.67	±.05	3	.60	±.13
4	.53	±.11	4,5	.38	±.21
5,6	.25	±.28			
Average	.623	±.040	Average	.54	±.10

The correlations are averaged by the formula

$$\frac{\Sigma(r/\sigma_r^2)}{\Sigma(1/\sigma_r^2)} \pm \sqrt{\frac{1}{\Sigma(1/\sigma_r^2)}}.$$

Thus 62 percent of the variability in the Beltsville data and 54 percent in the Chicago data is determined by factors common to litter-mates. The difference is not significant but as far as it goes it indicates less genetic variability in the Chicago data. In the Beltsville data, all of the substrain differentiation amounting to 18 percent of the total variance is of course included in that common to litter-mates. The variance common to litter-mates would be 54 percent $\left(=\frac{62-18}{100-18}\right)$ within a homozygous group or exactly that found in the Chicago data.

In the Chicago data comparisons have also been made between animals from different litters from the same mating. The results for successive litters and for litters two or more apart are shown below.

TABLE 6

Correlations between individuals of different litters from the same mating. Chicago stock.

		Following		
		3-T	4-T	Total
earlier	3-T	200	78	278
	4-T	85	66	151
	Total	285	144	429

r = +.25

TABLE 7

		2 or more later		
		3-T	4-T	Total
earlier	3-T	147	44	191
	4-T	124	81	205
	Total	271	125	396

r = +.29

The standard errors on the assumption of no correlation are .080 and .082 respectively. There is thus no doubt of the reality of these correlations. The average value .27 is just half that between litter-mates in the same material, indicating that factors which act alike on litter-mates but not on sibs from different litters, and factors which act alike on all offspring from the same mating contribute equally to variance in this stock in which there is little or no genetic variability. The parent-offspring correlation of .11 would indeed indicate a genetic fraternal correlation of .11 contributing to the .27 found above, but as will be brought out more fully later this figure is certainly too high. As to the nature of this group of factors, effects of the condition of the mother over long periods seem most probable.

VARIABILITY OF DIGITS OF ONE FOOT

All authors who have studied polydactyly of guinea pigs, have noted its frequent asymmetry. This in itself indicates an important element of variability which is not hereditary (WRIGHT 1916). The tables below show the relations between the left and right feet of the same animals in the Beltsville and Chicago records; applying the grades "poor" and "good" to single toes.

TABLE 8

Correlation between right and left feet of individuals.

			Right			
			3-T	POOR 4-T	GOOD 4-T	TOTAL
Left		3-T	1362	96	33	1491
	Poor	4-T	149	101	34	284
	Good	4-T	50	55	96	201
	Total		1561	252	163	1976

Beltsville
$r = +.78$

TABLE 9

			Right			
			3-T	POOR 4-T	GOOD 4-T	TOTAL
Left		3-T	206	26	3	235
	Poor	4-T	34	23	13	70
	Good	4-T	13	16	22	51
	Total		253	65	38	356

Chicago
$r = +.73$

On reducing these to 2×2 tables by grouping "poor" and "good" together, the tetrachoric correlations come out $+.779 \pm .015$ in the Beltsville data and $+.73 \pm .05$ in the Chicago data. The correlation within a homozygous substrain of the Beltsville stock should be about $.73\left(= \frac{.78 - .18}{1 - .18}\right)$, exactly that found in the Chicago data. This is another indication that genetic variability was practically lacking in the latter.

It is interesting to compare this correlation with that between *single* hind feet of litter-mates. The data below do this for 322 litter-mates of the Chicago data.

TABLE 10

The correlations between single feet of litter-mates of Chicago data.

		Left		
		3-T	4-T	TOTAL
Left	3-T	159	45	204
	4-T	46	72	118
	Total	205	117	322

$r = +.58$

TABLE 11

		Right		
		3-T	4-T	TOTAL
Right	3-T	337	72	409
	4-T	104	131	235
	Total	441	203	644

$r = +.59$

TABLE 12

		Right		
		3-T	4-T	TOTAL
Left	3-T	179	41	220
	4-T	42	60	102
	Total	221	101	322

$r = +.61$

There is obviously no special tendency toward resemblance of feet of the same side. The average, +.59, is surprisingly close to the correlation between the two feet of the same animal (+.73). Of the factors determining the condition of one foot, 27 percent act on that foot alone, 14 percent act on both feet of the individual but not on litter-mates, while 59 percent act alike on litter-mates.

Correlations of +.59 between single feet of litter-mates and of +.73 between feet of the same individual imply a correlation of $+.68\left(=\frac{4\times.59}{2\times1.73}\right)$ between the total factor complexes of litter-mates considered as the sum of those for the separate feet. The observed value +.54 is smaller probably because of lack of linearity in the relations of the factors for single toes and the scale for individuals based on the presence of at least one extra toe.

One point that comes out of both the Beltsville and Chicago data is the slightly greater frequency of little toes on the left side. Combining there were 26.0 percent on the left side and 22.2 percent on the right side. The difference is about 3 times its standard error. Both CASTLE and PICTET found an excess number of left digits.

SEX

There is no relation to sex. In the Beltsville data the percentage of polydactyls was 31.1 percent in the males and exactly the same to the first decimal place in females. In the Chicago data, there were 41.1 percent among the males and 42.4 percent among the females. This lack of difference contrasts with the important sex differences among otocephalic monsters and in amount of white spotting.

AGE OF DAM

With a large proportion of the variability common to litter-mates and only about half of this common to non-litter-mates from the same mating, it should be possible to find some indication from the records of the nature of the more important factors.

The most important relation to be found was one which was quite unexpected, namely with age of mother. Table 13 gives the results by months. The Beltsville inbred stock (1976 animals) is here combined with data from some selection experiments at Beltsville in which animals from family 35 were mated *inter se* without regard to relationship (246 animals) and with the Chicago data (358 animals). Figure 4 shows the percentage of polydactyls by month to 14 months and by such groupings of months thereafter as to keep the number in each group above 100. There is a rapid drop from the 83 percent and 60 percent produced by 3 and 4 month old females respectively to the 17 percent produced on the average by all females over 15 months of age. There is no question of the statistical significance by any method of calculation.

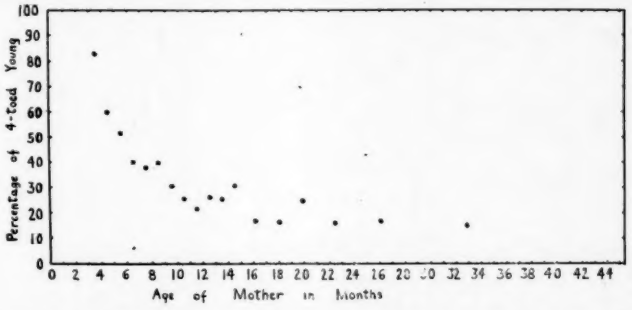

FIGURE 4.—Percentage of 4-toed young according to age of mother. All points except first (3 months) based on more than 100 young.

Furthermore, among the young which are 4-toed at all, the percentage of good polydactyls decreases rapidly with increasing age of the mother.

It is desirable to find whether these age effects are found in subdivisions of the family which differ markedly in total percentage of polydactyly. Tables 14–20 show the distributions in the same four divisions of the Beltsville inbred stock used previously, in the crossbred Beltsville stock, and in the Chicago stock as well as in the grand total, each by four age groups. In every case there is a significant downward trend of polydactyly in relation to age of mother. The squared correlation eta either for the Beltsville inbreds or the total is about .13, indicating 13 percent determination of the total variance by age of dam.

The effect has been presented as of age of mother rather than of father, but the Beltsville data give little basis for a distinction. Almost all of the brother-sister matings were between litter-mates and most of those in the miscellaneous group were between animals of nearly the same age. The exceptional cases however pointed to age of dam.

TABLE 13

Occurrence of polydactyly in relation to age of mother. Age 3 means from 3 months to 4 months, etc.

	AGE OF DAM IN MONTHS														
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3-toe	2	107	82	99	108	108	98	111	108	99	77	105	48	77	75
Poor 4-toe	3	123	71	56	55	65	41	30	21	35	27	42	6	19	8
Good 4-toe	7	39	16	10	11	7	2	8	8	1	—	6	—	—	—
Total	12	269	169	165	174	180	141	149	137	135	104	153	54	96	83
Percent 4-Toe	83.3	60.2	51.5	40.0	37.9	40.0	30.5	25.5	21.2	26.7	26.0	31.4	11.1	19.8	9.6

	AGE OF DAM IN MONTHS													
	18	19	20	21	22	23	24	25	26	27	28	29	30	31
3-toe	74	49	29	44	23	37	35	23	19	17	14	16	18	4
Poor 4-toe	17	9	15	9	5	3	7	6	3	5	1	3	4	2
Good 4-toe	4	2	—	1	—	2	—	—	—	—	—	—	—	1
Total	95	60	44	54	28	42	42	29	22	22	15	19	22	7
Percent 4-Toe	22.1	18.3	34.1	18.5	17.9	11.9	16.7	20.7	13.6	22.7	6.7	15.8	18.2	42.9

	AGE OF DAM IN MONTHS										
	32	33	34	35	36	37	38	39	40	45	TOTAL
3-toe	17	3	7	5	11	4	1	2	1	1	1758
Poor 4-toe	3	—	—	1	1	—	—	1	—	—	697
Good 4-toe	—	—	—	—	—	—	—	—	—	—	125
Total	20	3	7	6	12	4	1	3	1	1	2580
Percent 4-Toe	15.0	0.0	0.0	16.7	8.3	0.0	0.0	33.3	0.0	0.0	31.9

TABLES 14-20

Correlation of polydactyly with age of mother.

TABLE 14

Group I. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	53	75	104	108	340
Poor 4-toe	20	6	10	6	42
Good 4-toe	2	0	1	1	4
Total	75	81	115	115	386
Percent 4-toe	29.3	7.4	9.6	6.1	11.9

$\eta^2 = .11$, $\eta = .32$

TABLE 15

Group II. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	68	89	157	217	531
Poor 4-toe	28	33	40	30	131
Good 4-toe	8	2	4	0	14
Total	104	124	201	247	676
Percent 4-toe	34.6	28.2	21.9	12.1	21.4

$\eta^2 = .07$, $\eta = .27$

TABLE 16

Group III. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	29	41	106	131	307
Poor 4-toe	48	43	38	31	160
Good 4-toe	14	6	5	6	31
Total	91	90	149	168	498
Percent 4-toe	68.4	54.4	28.9	22.0	38.4

$\eta^2 = .18$, $\eta = .43$

TABLE 17

Group IV. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	15	29	73	67	184
Poor 4-toe	47	51	59	28	185
Good 4-toe	17	15	14	1	47
Total	79	95	146	96	416
Percent 4-toe	81.0	69.5	50.0	30.2	55.8

$\eta^2 = .20$, $\eta = .45$

TABLE 18

Beltsville crossbred.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	18	41	84	47	190
Poor 4-toe	21	12	12	4	49
Good 4-toe	3	3	1	0	7
Total	42	56	97	51	246
Percent 4-toe	57.1	26.8	13.4	7.8	22.7

$\eta^2 = .21$, $\eta = .45$

TABLE 19

Chicago.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	8	40	74	84	206
Poor 4-toe	33	31	36	30	130
Good 4-toe	18	2	1	1	22
Total	59	73	111	115	358
Percent 4-toe	86.4	45.2	33.3	27.0	42.5

$\eta^2 = .25$, $\eta = .50$

Percent 4-Toe 31.9 0.0 0.0 33.3 0.0 0.0 8.3 16.7 0.0 0.0 15.0

TABLE 20

Grand Total

	AGE OF DAM (MONTHS)				TOTAL
	3–6	6–9	9–15	15–	
3-toe	191	315	598	654	1758
Poor 4-toe	197	176	195	129	697
Good 4-toe	62	28	26	9	125
Total	450	519	819	792	2580
Percent 4-toe	57.6	39.3	27.0	17.4	31.9
Percent good of 4-toe	23.9	13.7	11.8	6.5	15.2
Percent good of total	13.8	5.4	3.2	1.1	4.8

$\eta^2 = .134$, $\eta = .366$

In the Chicago experiments an especial effort was made to mate young females with old males and *vice versa*. Table 21 shows the results classified in 4 age groups for each parent. A relation to age of mother comes out as definitely as in the Beltsville data ($\eta = .50$). The slight downward

TABLE 21

Occurrence of 3-toed and 4-toed young in relation to ages of sire and of dam. Chicago data.

AGE OF SIRE	AGE OF DAM								TOTAL		
	3–6		6–9		9–15		15–37		3-T	4-T	PERCENT 4-T
	3-T	4-T	3-T	4-T	3-T	4-T	3-T	4-T			
3–6	0	10	2	2	6	2	8	4	16	18	52.9
6–9	2	4	4	12	7	6	18	7	31	29	48.3
9–15	4	18	20	8	30	9	20	9	74	44	37.3
15–37	2	19	14	11	31	20	38	11	85	61	41.8
Total	8	51	40	33	74	37	84	31	206	152	42.5
Percent 4-toe	86.4		45.2		33.3		27.0		42.5		

trend in relation to age of sire in part reflects a slight correlation with age of dam present even in this data. It is not statistically significant. It is probable the entire effect is one of age of dam.

Table 22 shows the percentage of 4-toed young in each of the 4 subdivisions of the Beltsville data in relation to the age of the grandparents at birth of the parents (or dam in the few cases in which the parents were not litter-mates). There appears to be a slight downward trend of the percentage in the totals (next to last column) but this practically disappears (last column) on correcting for the irregularities in distribution among the 4 groups by weighting the percentages by the totals for each group. Correction for age of dam was tried but made no appreciable change in the re-

TABLE 22

Occurrence of polydactyly in relation to age of dam's parents. Beltsville.

AGE OF GRANDAM	SUBSTRAIN (BELTSVILLE)								TOTAL		
	I		II		III		IV		NUMBER	PERCENT 4-T	WEIGHTED PERCENT 4-T
	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T			
3–5	94	7.4	174	25.3	128	43.0	129	58.1	525	34.5	33.2
6–8	91	13.2	106	14.2	88	38.6	114	49.1	399	29.3	27.5
9–14	69	14.5	211	22.7	167	38.3	122	59.0	569	34.1	32.7
15–45	132	12.9	185	20.5	115	33.0	51	56.9	483	25.3	29.8
Total	386	11.9	676	21.4	498	38.4	416	55.8	1976	31.1	31.1

sult. There is thus no reliable indication of any persistence of the effect beyond the immediate progeny of immature females. A direct physiological effect of age of mother on the egg or embryo is indicated.

TABLE 23

Polydactyly (D) in relation to age of dam (A) and birth rank (B) in Beltsville data.

BIRTH RANK	AGE OF DAM													
	3, 4		5		6–9		9–15		15–21		21–45		TOTAL	
	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T
1	212	57.5	137	45.3	72	51.4	—	—	—	—	—	—	421	52.5
2	—				317	37.5	72	25.0					389	35.2
3					1	0.0	304	30.3	20	5.0			325	28.6
4, 5							235	26.0	226	19.9	18	0.0	479	22.1
6–12									84	17.9	278	15.1	362	15.7
Total	212	57.5	137	45.3	390	40.0	611	28.0	330	18.5	296	14.2	1976	31.1

There is a possibility that the effect may be one of birth rank rather than age of dam, since these were closely correlated. Table 23 shows the relations in the Beltsville stock. There is a somewhat closer relation of polydactyly to age of dam ($\eta_{DA} = -.360 \pm .027$) than to parity ($\eta_{DB} = -.343 \pm .027$) with a correlation of $+.922 \pm .034$ between age and parity. The correlation of polydactyly with parity is reduced to the insignificant figure $-.030 \pm .030$ by holding age of dam constant while the correlation with age remains significant ($-.121 \pm .030$) (correcting an error in a previously published figure, WRIGHT 1926). It is concluded that parity has no effect on occurrence of polydactyly by itself.

TABLE 24

Occurrence of 3-toed and 4-toed young in relation to age and weight of dam.

WEIGHT OF DAM (GRAMS)	AGE OF DAM (CHICAGO)								TOTAL		
	3–6		6–9		9–15		15–37				
	3-T	4-T	3-T	4-T	3-T	4-T	3-T	4-T	3-T	4-T	PERCENT 4-T
400	0	2							0	2	
450	1	2	1	2	3	1			5	5	61.5
500	3	15	3	0	2	0	2	2	10	17	
550	2	12	12	6	5	1	2	0	21	19	47.5
600	1	18	9	5	12	7	9	2	31	32	50.8
650			8	6	20	8	12	3	40	17	29.8
700			2	9	29	11	26	9	57	29	33.7
750			2	2	3	4	15	4	20	10	
800					0	5	3	1	3	6	37.8
850							5	1	5	1	
Total	7	49	37	30	74	37	74	22	192	138	41.8
Percent 4-toe	87.5		44.9		33.3		22.9				

It is possible that the weight of the dam is more important than her age. The weights at the time of birth of the young were taken in the Chicago data. The relation to polydactyly and to age of dam are shown in table 24. It is evident from the marginal totals that the occurrence of polydactyly is much less closely correlated (negatively) with weight ($r = -.23 \pm .07$) than with age ($r = -.53 \pm .05$). The correlation between weight and age of dam is $+.58$ and the partial correlation of polydactyly with weight, for constant age comes out with reversed sign $+.11 \pm .07$. On the whole it is the heavier (and hence more rapidly growing) females of a given age class, rather than the lighter ones which produce the highest percentage of polydactyls although the relation is of doubtful significance.

TABLE 25

The averages of various characters at different ages of the dam. The standard deviation of size of litter was 1.01, for birth weight 16.5 reduced to 12.8 for constant size of litters (correlation −.63), for weaning weight 45.5 reduced to 38.5 for constant size of litter (correlation −.53), and for percentage of white 18.9 percent.

AGE OF DAM	NUMBER	PERCENT 4-T	LITTER SIZE	PERCENT ♂	PERCENT BORN DEAD	PERCENT DIED OF LIVE BORN	BIRTH WEIGHT	33-DAY WEIGHT	PERCENT WHITE MALES	PERCENT WHITE FEMALES
3–6	349	52.7	2.45	53.3	27.8	22.5	79.9	221.6	56.3	60.5
6–9	390	40.0	2.37	50.8	20.2	21.2	79.8	225.0	59.5	67.6
9–12	319	29.2	2.63	48.8	18.6	20.5	80.9	220.9	60.6	66.5
12–15	292	26.7	2.41	54.7	19.4	17.8	83.0	228.3	61.3	70.6
15–21	330	18.5	2.52	54.0	21.3	23.3	80.2	224.7	63.2	69.6
21–45	296	14.2	2.30	49.2	24.1	19.4	83.5	241.1	66.9	73.3
Total	1976	31.1	2.44	51.8	22.0	20.9	81.0	226.5	61.2	67.8

It seemed likely that a study of other effects of the age of the mothers might throw light on the unexpected relation to percentage of polydactyly. Table 25 presents a summary of the principal results in 6 age classes chosen to include approximately equal numbers. Except for an equally unexpected effect on the percentage of white in the young, there is remarkably little indication of age effects. Size of litter is at its highest in females which are approaching or have just reached maturity and lowest in the oldest group. There is a decreasing percentage of stillborn young with increasing maturity but a rise in the oldest group makes this difficult to relate to polydactyly. Mortality between birth and weaning shows nothing significant. The birth weight of young born alive and weaning weight (both corrected for litter size by the factors given by WRIGHT and EATON, 1929) show some tendency to increase with increasing age of the mother but the trends are slight and hardly significant.

The females do not reach full size until 15 months of age. On the whole it appears that the offspring from immature females are at some disadvantage, but this is very slight. The point established by these comparisons is that immaturity of the mother has a much greater influence on the development of an atavistic little toe by the young than on a number of characters, in which an effect would seem more likely on *a priori* grounds.

CORRECTIONS FOR AGE OF DAM

The important effect of age of dam makes it necessary to test whether other apparent effects may not be merely consequences of an irregular age distribution.

TABLE 26

The results of different types of matings classified by age of dam. Beltsville.

DAM	SIRE	AGE OF DAM (BELTSVILLE) 3–6 NUMBER	3–6 PERCENT 4-T	6–9 NUMBER	6–9 PERCENT 4-T	9–15 NUMBER	9–15 PERCENT 4-T	15– NUMBER	15– PERCENT 4-T	TOTAL NUMBER	TOTAL PERCENT 4-T	WEIGHTED PERCENT 4-T
3-toe	3-toe	198	42.9	209	34.4	337	20.5	275	16.4	1019	26.6	25.9
3-toe	4-toe	39	66.7	55	34.5	53	35.8	38	26.3	185	40.0	38.0
4-toe	3-toe	36	50.0	37	32.4	66	25.8	131	9.9	270	22.2	26.3
4-toe	4-toe	76	72.4	89	59.6	155	42.6	182	19.2	502	41.6	43.8
Total		349	52.7	390	40.0	611	28.0	626	16.5	1976	31.1	

Table 26 shows that correction for age distribution in the Beltsville stock has little effect on the production of different types of mating (last two columns). There is some increase in the regularity of the results. In the Chicago stock on the other hand, it happened that matings of 4-toe×4-toe produced very few young in the last age classes. On weighting the per-

TABLE 27

The results of different types of matings, classified by age of dam. Chicago.

DAM	SIRE	AGE OF DAM (CHICAGO) 3–6 NUMBER	3–6 PERCENT 4-T	6–9 NUMBER	6–9 PERCENT 4-T	9–15 NUMBER	9–15 PERCENT 4-T	15– NUMBER	15– PERCENT 4-T	TOTAL NUMBER	TOTAL PERCENT 4-T	WEIGHTED PERCENT 4-T
3-toe	3-toe	17	88.2	31	35.5	44	34.1	48	35.4	140	41.4	43.7
3-toe	4-toe	14	64.3	9	44.4	19	21.1	29	13.8	71	29.6	30.6
4-toe	3-toe	9	100.0	11	36.4	20	45.0	30	23.3	70	41.4	45.3
4-toe	4-toe	19	94.7	22	63.6	28	32.1	8	37.5	77	57.1	50.6
Total		59	86.4	73	45.2	111	33.3	115	27.0	358	42.5	
3-toe parent		57	84.2	82	36.6	127	33.9	155	29.0	421	39.4	41.2
4-toe parent		61	88.5	64	56.3	95	32.6	75	22.7	295	46.8	43.5

centage of polydactyls from each type of mating and age class by the total number from that age class, the evidence for heredity largely disappears. On combining males and females to increase the number it appears that 3-toed parents produced 41.2 percent polydactyls and 4-toed parents only slightly more, 43.5 percent, although there was some tendency to mate 3-toe with 3-toe and 4-toe with 4-toe. The tetrachoric correlation between parent and offspring from this data is only +.03 instead of +.11. This result, in conjunction with the reduced correlation between litter-mates and between right and left foot in the data lead to the conclusion that genetic variation is practically absent in this group descended from a single mating in the 22nd generation of brother-sister mating.

SEASONAL EFFECTS

The Beltsville records of family 35 were tabulated by month of birth. Table 28 shows the percentage of 4-toed young in each month.

TABLE 28

Percentage of polydactyls by months of birth. Beltsville.

MONTH OF BIRTH	NUMBER	PERCENT 4-T	MONTH	NUMBER	PERCENT 4-T
May	136	28.7	November	161	32.3
June	160	24.4	December	159	30.2
July	231	24.2	January	160	44.4
August	176	29.0	February	149	39.6
September	140	24.3	March	148	43.9
October	215	23.7	April	141	34.8
May–October	1058	25.5	November–April	918	37.5

There is an unquestionably significant cycle with respect to polydactyly. The χ^2 test (24 classes, 11 degrees of freedom, $\chi^2=48$) yields a probability of .000002 that the differences can be due to random sampling with no allowance for the grouping of signs. But in every month from May to October there were less than 30 percent polydactyls (average 25.5 percent) and in every one of the remaining 6 months there were more than 30 percent (average 37.5 percent). A difference in average is shown within each of the four groups (table 29) into which the family has been divided and also from dams of each age class (table 30). This seasonal cycle accounts for about 3.5 percent of the total variance.

TABLE 29

Percentage of polydactyls in two 6-months periods in each group of substrains. Beltsville.

MONTHS OF BIRTH	GROUPS OF SUBSTRAINS							
	GROUP I		GROUP II		GROUP III		GROUP IV	
	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T
May–October	214	10.3	356	18.3	362	28.6	226	47.8
November–April	172	14.0	320	25.0	236	49.2	190	65.3

TABLE 30

Percentage of polydactyls in two 6-month periods at different ages of the dams. Beltsville.

MONTHS OF BIRTH	AGE OF DAM							
	3–6		6–9		9–15		15–45	
	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T
May–October	157	46.5	224	37.1	346	23.4	331	10.0
November–April	192	57.8	166	44.0	265	34.0	295	23.7

It is somewhat disconcerting therefore to find that in the Chicago data the cycle is reversed. There were 51.5 percent polydactyls born from May to October and only 34.6 percent from November to April. This difference is 3.2 times its standard error on the basis of the numbers of young in the two periods, but becomes somewhat doubtful on allowing for the intra-litter correlation of +.54. This result can not, of course, invalidate the significance of the Beltsville cycle but makes it clear that it is not season *per se* which is responsible.

Table 31 shows the averages of a number of characters in the two 6-month periods which are most significant for polydactyly. A tabulation by

TABLE 31

Averages of various characters in two 6-month periods. Beltsville. Birthweight is of liveborn young.

MONTHS OF BIRTH	NUMBER	PERCENT 4-T	SIZE OF LITTER	PERCENT BORN DEAD	DIED OF LIVEBORN	BIRTH WEIGHT	33 DAY WEIGHT	PERCENT WHITE ♂	PERCENT WHITE ♀
May–October	1058	25.5	2.50	21.1	17.8	80.7	229.8	61.1	68.5
November–April	918	37.5	2.38	23.0	24.4	81.5	222.5	61.3	67.0
Total	1976	31.1	2.44	22.0	20.9	81.1	226.6	61.2	67.8

months indicates that this division of the year is as significant as any in the other cases. It is remarkable that polydactyly should show a more marked seasonal variation (in the Beltsville data) than such characters as birth weight and percentage of stillborn (neither of which showed any appreciable cycle during this period) or size of litter, percentage of mortality between birth and weaning and weight at weaning. However, the litters were smaller, the postnatal mortality higher and the weaning weight lower during the period (November to April) in which the percentage of polydactyls was high, as compared with the remaining 6 months. On the whole polydactyly appears to be associated with unfavorable conditions. The percentage of white in the coat is included in table 31 as a character which agreed with polydactyly in showing an important effect of age of dam, but it shows no seasonal cycle in the same animals in which polydactyly shows a marked cycle. Even size of litter and the mortality percentages give no indication of a cycle in the Chicago data in which the cycle for polydactyly seems to have been reversed.

The most important difference between the Beltsville and Chicago conditions was probably in the green feed. There was a rather definite feeding cycle at Beltsville: freshly cut grass and green alfalfa from late spring to mid-fall, the period in which relatively few polydactyls were born; stored cabbage and kale, often inadequate in quantity or quality during the rest of the year when many polydactyls were born. Stored cabbage has been used throughout the year at Chicago.

About all that can be said is that some environmental factor, generally unfavorable, but with a relatively specific tendency to induce polydactyly, varied seasonally under the conditions at one laboratory, but is not inevitably associated with season as shown by results in another laboratory.

SIZE OF LITTER

Table 32 shows the percentages of 4-toed young in each size of litter. There are rather striking differences in the Beltsville data and ones which appear to be statistically significant even if litters instead of individuals

TABLE 32

Percentage of polydactyls among young born in each size of litter. Beltsville and Chicago.

SIZE OF LITTER	BELTSVILLE		CHICAGO	
	NUMBER YOUNG	PERCENT 4-T	NUMBER YOUNG	PERCENT 4-T
1	150	22.7	29	37.9
2	562	34.5	94	34.0
3	795	34.8	152	46.7
4	369	26.3	68	41.2
5, 6	100	12.0	15	66.7
Total	1976	31.1	358	42.5

are made the units. It is, however, difficult to interpret a high percentage in litters of 2 and 3 and low percentages in litters of 1, 4 and 5. The Chicago data do not confirm the order in the Beltsville data. About all that can be said at present seems to be that there are relations of some sort between factors which affect size of litter and ones which affect number of digits.

COMPARISONS OF 3-TOED AND 4-TOED INDIVIDUALS

There is not much difference in the characteristics of 3-toed and 4-toed individuals apart from digit number. The Beltsville data show a lower percentage of 4-toed born dead than of 3-toed (15.8 percent *vs.* 22.9 percent). Not much confidence can be placed in this, however, since it is possible that a rudimentary little toe was overlooked more frequently in the young born dead than in those born alive in these records in which digit numbers were recorded merely as a matter of routine in a large colony of which family 35 constituted only a small portion. In the Chicago data the feet were examined with the greatest care in all stillborn animals of family 35 and no significant relation was found (30.3 percent born dead among polydactyls and 33.0 percent among normals).

The percentage of liveborn which died between birth and weaning was higher among polydactyls in the Beltsville data (34.2 percent *vs.* 19.2 percent). There is no reason for any systematic error in the routine records here, since these animals were all alive when recorded. There is an indication here that the factors which determine polydactyly are otherwise unfavorable, confirming previous indications. The parallelism in the seasonal cycles of polydactyly and this mortality percentage of course contributes a little (about 0.9 percent) to the above difference. The Chicago data show no important difference between 4-toed and 3-toed in this respect (22.6 percent of the 4-toed and 21.7 percent of the 3-toed died before weaning).

The average birth weight of the 4-toed young born alive (81.2 grams) was practically the same as for 3-toed young (81.0 grams). The 4-toed,

however, had the advantage of being born in slightly smaller litters (average 2.72) than 3-toed (2.84). Correction for the regression of −11.3 grams per unit change in litter size brings the birth weight of 4-toed below that of 3-toed but hardly to a significant extent (difference 1.1 grams).

The average weights at weaning show more difference: 4-toed 221.4 grams, 3-toed 228.8 grams. The difference is increased to about 10.0 grams on allowing for the regression of −26.2 grams per unit change in litter size. This appears to be statistically significant, having a standard error of about 2.4 grams on the basis of a standard deviation of 38.5 grams (corrected for size of litter) and the number of animals involved (848 3-toed, 392 4-toed). It is significant even on increasing some 25 percent to allow for the intra-litter correlations of the characters involved. The parallelism in seasonal cycle contributes very little to this difference. Again there is an indication that the factors of polydactyly are on the whole of an injurious sort. More important, however, is the conclusion that the physical differences between 3-toed and 4-toed representatives of family 35 are of a trivial sort. The environmental factors determining polydactyly must be for the most part rather specific for this character.

SUMMARY

The recurrence of an atavistic little toe is not uncommon in strains of guinea pigs. Among 23 inbred families, 12 never produced 4-toed young, 5 produced a few sporadics, while the remaining 6 produced percentages ranging from 2 to 19 (1906 to 1915). A branch of one of these, derived from a single mating in the 12th generation of brother-sister mating produced 31 percent at Beltsville, Maryland. Subdivisions of this ranged from 9 percent to 69 percent. A large random-bred group derived from a single mating of this family in the 22nd generation, produced 42 percent (in Chicago).

It is assumed that the presence or absence of the little toe depends on whether the combination of factors exceeds or falls below a threshold. On a scale of uniform factor effects, 18 percent of the variance of the Beltsville stock was genetic, due to substrain differences (squared biserial eta .18, also parent-offspring correlation .18). There was no demonstrable genetic variability within substrains. Genetic variability was also negligible in the Chicago stock (parent-offspring correlation .03, after correction for other factors).

Litter-mates were determined 62 percent by common factors in the Beltsville stock (correlation +.62). This includes the genetic factors and 54 percent of the non-genetic ones. In exact agreement is the correlation of .54 between litter-mates in the Chicago stock.

The non-genetic factors common to litter-mates are equally divided be-

tween ones also common to non-litter-mates from the same mating (correlation .27) and ones common only to litter-mates (Chicago stock). These results lead to the following analysis of variability.

	BELTSVILLE	CHICAGO
Hereditary differences (substrains)	18	0
Environmental differences common to		
Sibships	22	27
Litters	22	27
Individual	38	46
Total	100	100

There is frequent asymmetry. The correlation between left and right was .78 in the Beltsville data and .73 in the Chicago data. That between single feet of litter-mates was .59 in the Chicago data. These results in combination with the preceding lead to the following analyses of the factors responsible for digit numbers on a single foot.

	BELTSVILLE	CHICAGO
Hereditary differences	19	0
Environmental differences common to		
Sibships	24	29.5
Litters	24	29.5
Individuals	11	14
Foot	22	27
Total	100	100

Part of the variance assigned to the individual in the analysis of individual variability is spurious due to non-additive effects. The analysis of the variance of single feet is more satisfactory.

No direct evidence was obtained of the nature of the non-genetic factors affecting whole sibships. Presumably long-continuing conditions of the mother are involved.

The most important factor affecting whole litters is age of the mother. This determined 13 percent of the total variance in the Beltsville stock and (less reliably) 25 percent in the Chicago stock. Immature females produce much higher percentages of 4-toed young than mature females. The effect is primarily neither one of parity nor of weight of mother. Indeed the heavier females of a given age produce slightly more polydactyls than the lighter ones. A physiological competition for some substance between growing mother and embryo seems the most plausible suggestion.

A seasonal cycle, high percentage of polydactyls in late fall, winter and early spring, low the rest of the year, accounted for 3 percent of the total variance in the Beltsville stock. That this was not an effect of season *per se* was indicated by a reversal in the Chicago stock. The seasonal effect and relation to rate of growth and death rate, indicate that unfavorable conditions tend to increase the percentage of polydactyls. The 4-toed individuals are not, however, at all handicapped in prenatal growth or chances of live birth and only slightly in postnatal growth and viability.

No direct evidence has been obtained of the nature of the individual factors. The extremely local action of important factors is indicated by the frequent asymmetry. In this the left foot is slightly more likely than the right to develop a little toe. There is no relation to sex.

A recognition and evaluation of the importance of non-genetic factors in determining the presence or absence of the little toe is a necessary foundation for analysis of the genetic differences between different stocks.

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THE RESULTS OF CROSSES BETWEEN INBRED STRAINS OF GUINEA PIGS, DIFFERING IN NUMBER OF DIGITS

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Received January 12, 1934

INTRODUCTION

Guinea pigs normally have only 3 toes on the hind feet. This is the condition in all of the wild species of the family Caviidae. Atavistic return of the little toe is not, however, especially uncommon. CASTLE (1906) noticed an animal with an imperfect little toe and by inbreeding and selection developed a strain in which every animal had 4 perfect digits on the hind feet. Strain D, in my possession, traces exclusively to 3 animals of this strain, kindly presented by Professor CASTLE in 1915. It has bred true to the 4-toed condition to the present time. As there have seldom been more than 2 or 3 breeding males at a time, there has been much inbreeding. It may safely be assumed to be homozygous in most respects.

CROSSES BETWEEN STRAINS 2 AND D

Family 2 is a strain tracing by exclusive brother-sister mating to a single pair mated in 1906 in an inbreeding experiment of the U. S. Bureau of Animal Industry (WRIGHT 1922a). Two 4-toed animals were recorded from it in its early history among about 3,500 young. All of the animals used in the crossbreeding experiments described here traced to a single mating in the sixth generation, which has had only 3-toed descendants. Those used in the Chicago series of experiments all traced to a single mating in the 15th generation. This strain may safely be assumed to be homozygous or nearly so in some combination of genes which determines the normal 3-toed condition of the hind feet.

Reciprocal crosses were made between strains D and 2 at Beltsville, Maryland. (U.S. Bureau of Animal Industry) in 1923–25. Female D × male 2 produced 11 young, all normal. Female 2 by male D produced 76 young, also all normal. In experiments in Chicago, 1926–30, matings of female 2 by male D produced 59 young, again all normal. Thus F_1 included a total of 146 young, all of which were 3-toed. The results in F_2 were as follows. No separation is made by sex in this and later tables. Separate tabulations of males and females have been made but in no case do they show any significant difference. In these tables good 4-toe means that the little toes of both hind feet were of maximum size and too firm to be bent easily back to the foot.

TABLE 1

Results of F_2 from matings of the pure 3-toed stock 2 with the pure 4-toed stock D.

F_2(2×D)	PERCENTAGE			TOTAL NUMBER
	3-TOE	POOR 4-TOE	GOOD 4-TOE	
Beltsville	81.6	9.2	9.2	207
Chicago	73.1	23.1	3.8	26
Total	80.7	10.7	8.6	233

If all polydactyls be combined the proportion, 19.3 percent, differs from 25 percent by only 2.0 times the standard error, an amount which is hardly significant. The first suggestion from the results in F_1 and F_2 is thus that polydactyly depends on one major recessive factor, supplemented by modifiers necessary for perfect development of the little toe and occasionally, perhaps, for expression of the digit at all.

The results of backcrosses of F_1 females to males of the pure 4-toed stock D, appear to confirm this interpretation.

TABLE 2

Results of backcross, female 3-toe from (2×D) with males of pure-toed stock D. Young 3/4 blood D.

	FEMALE (2×D)	MALE (D)	PERCENTAGE			TOTAL NUMBER
			3-TOE	POOR 4-TOE	GOOD 4-TOE	
Beltsville	3-toe	good 4-toe	45.1	20.7	34.2	82
Chicago	3-toe	good 4-toe	44.4	23.7	31.9	207
Total	3-toe	good 4-toe	44.6	22.8	32.5	289

The proportion of 3-toed young does not differ significantly from 50 percent (1.8 times standard error).

There are many cases in slow-breeding animals in which a gene has been designated on no more basis than dominance in F_1 of a cross between true breeding strains, a 3:1 ratio in F_2 and a 1:1 ratio in the backcross to the recessive strain. There is, however, no conclusive evidence for or against its existence until breeding tests have been made of the segregating generation. In the present case such tests were made by mating all types which came from the backcross matings (Chicago series) to the pure 4-toed strain, D. Only females were tested in this and later generations because of the very low fertility of the D females, which made it desirable to use all that were reared in maintaining the pure D stock.

The supposed dominants (3-toed) produced only 22.6 percent 3-toed young, significantly less than the expected 50 percent on the basis of one major factor.

TABLE 3

Results of 3 top crosses of pure 4-toed stock (D) on strain 2. Young 7/8 blood D.

FEMALE ((2×D)×D)	MALE (D)	PERCENTAGE			TOTAL NUMBER
		3-TOE	POOR 4-TOE	GOOD 4-TOE	
3-toe	Good 4-toe	22.6	32.2	45.2	186
Poor 4-toe	Good 4-toe	34.5	13.8	51.7	29
Good 4-toe	Good 4-toe	10.0	24.4	65.6	90

Even more disconcerting was the result from the supposed recessives (good and poor 4-toed combined) which mated with pure 4-toed produced as many as 16.0 percent 3-toed. There is indeed no significant difference (1.4 standard error) between this 16.0 percent normals from 4-toed mothers and the 22.6 percent from 3-toed mothers. It may be added that matings between perfect polydactyls of the backcross generation produced 2 3-toed, 3 poor 4-toed and 5 good 4-toed young.

Clearly there is no one major factor distinguishing strains D and 2. Perhaps there are two factors of about equal importance. In this case 25 percent of the backcross progeny should be homozygous in both. Most of the good polydactyls of this progeny should be of this type since these made up only a slightly larger percentage (32.5 percent). Yet even these when tested by mating with the pure 4-toed strain produced 10 percent 3-toed young as well as 24 percent with rudimentary little toes. Ten females were tested in this way. Nine of them produced 3-toed or poor 4-toed young and the other had only two young altogether. There is thus no evidence that any of the *good* 4-toed animals of the backcross progeny bred like the animals of the pure 4-toed stock which they resembled phenotypically. There must be more than two equally important factors distinguishing strains 2 and D.

With three factors, 12.5 percent of the backcross progeny and thus nearly half of those with well-developed little toes, should be homozygous for all three. The fact that 9 out of 10 were proved to be genetically different from strain D (the other inadequately tested) practically rules out this hypothesis. It may be concluded that strain D and 2 differ in at least 4 factors of comparable importance.

There is indeed very little evidence of any genetic differentiation in the entire backcross progeny. There is a remarkable approach to completely blending inheritance of a character which approaches alternative expression. On the other hand, the appearance of polydactyls in F_2 after their complete absence in F_1 indicates that there really is segregation and that the number of genes is not indefinitely large.

An attempt to obtain further evidence of segregation was made by testing females of the second backcross progeny by yet another backcross to the pure 4-toed stock.

TABLE 4

Results from four top crosses of pure 4-toed stock (D) on strain 2. Young 15/16 blood stock D.

FEMALES (((2×D)×D)×D)×D)	MALE (D)	PERCENTAGE			TOTAL NUMBER
		3-TOE	POOR 4-TOE	GOOD 4-TOE	
3-toe from 3-toe	Good 4-toe	16.7	43.3	40.0	60
Good 4-toe from 3-toe	Good 4-toe	0	8.7	91.3	69
Good 4-toe from good 4-toe	Good 4-toe	0	0	100.0	28

There are real enough differences here. Two successive selections of perfect polydactyly are enough to give females which breed like those of the pure 4-toed strain as far as this test goes. But perfect polydactyls selected from the progeny of 3-toed mothers produced 9 percent imperfect polydactyls even though their offspring were 15/16 blood of strain D. Three-toed females from 3-toed mothers produced almost the same results from the mating with strain D as did their mothers. The percentage of perfect polydactyls is actually less (40 percent instead of 45 percent) though not to a significant extent. It appears that a point has been reached at which the swamping effect of repeated backcrossing to the pure 4-toed stock can be neutralized by counter selection.

Another generation of such backcrossing was attempted. Unfortunately, the very low fertility of the females of the D stock had come to be characteristic of these later backcross generations. No young were obtained from the 3-toed females of this generation and only a few from the others.

TABLE 5

Results of 5 top crosses of pure 4-toed stock (D) on strain 2. Young 31/32 blood stock D.

FEMALES ((((2×D)×D)×D)×D)×D)	MALES (D)	PERCENTAGE			TOTAL NUMBER
		3-TOE	POOR 4-TOE	GOOD 4-TOE	
Poor 4-toe from 3-toe from 3-toe	Good 4-toe	0	25.0	75.0	12
Good 4-toe from 3-toe from 3-toe	Good 4-toe	0	0	100.0	6
Good 4-toe from good 4-toe from good 4-toe	Good 4-toe	0	0	100.0	7

The young here are 31/32 blood of pure 4-toed stock, yet it has been possible to maintain rudimentary development of the little toe by counter selection. This confirms the results from F_2 and the preceding backcross generation that there is segregation of a not indefinitely large number of factors.

CROSSES BETWEEN STRAINS 32 AND D

Before attempting further interpretation of the results of this cross, it will be well to present data from crosses between strain D and other inbred

strains. Family 32, like 2, was started in 1906 from a single pair and maintained by exclusive brother-sister mating by the U.S. Bureau of Animal Industry. All of the recorded young were 3-toed. Females descended from a single mating in the 11th generation were mated with males of strain D and the young were mated *inter se* or backcrossed to strain D. The results were remarkably similar to those from the experiments with strain 2, with one exception. In F_1, there was a single imperfect polydactyl (one feeble extra toe on one foot) to 25 3-toed young. The similarity of the results in F_2 and the backcross progeny makes it probable that the F_1 from 2×D was also not far below the threshold for polydactyly in spite of the failure of any to appear among 146 young.

TABLE 6

Results of cross between 3-toed strain 32 and pure 4-toed strain D, of F_2 and of backcross of F_1 to strain D.

	FEMALE	MALE	PERCENTAGE			TOTAL NUMBER
			3-TOE	POOR 4-TOE	GOOD 4-TOE	
F_1	3-toe (Fam. 32)	4-toe (D)	96.2	3.8	0	26
F_2	3-toe (32×D)	3-toe* (32×D)	80.0	6.0	14.0	50
BX	3-toe (32×D)	4-toe D	45.8	25.0	29.2	24

* The single poor 4-toed F_1, a male, is included. He produced 2 3-toed and 1 good 4-toed.

CROSSES BETWEEN STRAINS 13 AND D

Family 13 also was started in 1906 from a pair and maintained by brother-sister mating in the experiments of the U.S. Bureau of Animal Industry. One polydactyl has been recorded among some 6,000 young. This was otherwise abnormal, being clubfooted, a very uncommon condition in family 13.

The cross breeding experiments were made with animals descended from a single mating in the 9th generation. They may be assumed to be homozygous in most factors.

The results are very different from those obtained by mating the same 4-toed strain with the other two 3-toed strains (2 and 32). About one-third of F_1 were polydactyls. The reciprocal crosses are tabulated separately, but there is no significant difference, giving further evidence that there is equal transmission by males and females. That the appearance of polydactyls in F_1 is not a result of segregation is clearly shown by the results in F_2 and in the backcrosses to the pure 4-toed strain. Thus, F_2 from 3-toed ×3-toed actually included fewer 3-toed and more good 4-toed young than did F_2 from matings between 4-toed F_1's, although the differences are not significant. In the backcross tests the 3-toed F_1's produced somewhat fewer good 4-toed (but more poor 4-toed) than did the 4-toed F_1's tested

TABLE 7

Results of crosses between 3-toed strain 13 and 4-toed strain D, of F_2 and of backcross of F_1 to strain D.

	FEMALE	MALE	PERCENTAGE			TOTAL NUMBER
			3-TOE	POOR 4-TOE	GOOD 4-TOE	
F_1	3-toe (13)	4-toe (D)	63.6	36.4	0.0	44
	4-toe (D)	3-toe (13)	73.9	26.1	0.0	23
F_2	3-toe (F_1)	3-toe (F_1)	42.9	19.0	38.1	84
	4-toe (F_1)	4-toe (F_1)	53.1	18.8	28.1	32
BX	3-toe (F_1)	4-toe (D)	16.7	33.3	50.0	12
	4-toe (F_1)	4-toe (D)	13.2	25.0	61.8	68
Total F_1			67.2	32.8	0.0	67
Total F_2			45.7	19.0	35.3	116
Total Backcross			13.8	26.2	60.0	80

in the same way, but again the differences are not significant. The variability in F_1 must therefore be attributed to non-genetic factors similar to those demonstrated within another inbred strain (35) in the preceding paper of this series.

Turning to the totals for F_1, F_2 and the backcross generation, it will be seen that the simulation of one-factor Mendelian heredity completely breaks down in this case. Clearly family 13, though equally as normal as families 2 and 32, is closer to the threshold for polydactyly.

CROSSES BETWEEN STRAINS 35 AND D

Family 35 also was started from a pair in 1906 and has been maintained by brother-sister mating by the U.S. Bureau of Animal Industry. It has produced a high percentage of polydactyls in most of its branches. An analysis of its record was the subject of the first paper of this series. It was shown that a large branch descended from a single mating in the 12th generation, broke up into substrains ranging in extreme cases from 9 percent to 69 percent incidence of polydactyly but that the variability due to this cause made up only 18 percent of the total variance. The non-genetic variance (82 percent) was largely due to factors common to litter-mates (44 percent), leaving 38 percent of the total as due to separate action on individuals. Age of mother was the most important factor common to litter-mates to be demonstrated. All genetic variability appeared to have been lost in the strain maintained in Chicago descended from a single mating in the 22nd generation. In this case the variability was analyzed into 3 components, that common to whole sibships (27 percent), that common to litter-mates but not sibships (27 percent) and that which was individual in incidence (46 percent).

This analysis of variance was based on the assumptions that the alternative categories, 4-toed and 3-toed, depend on whether the combination of factors, genetic and non-genetic, is above or below a certain threshold, and that there is a normal distribution on this hypothetical scale of factor combinations.

The records of the family in Beltsville from the 12th generation and in Chicago from the 22nd generation are given below with the results of crosses with the pure 4-toed stock D made at Beltsville. Note that F_1 was backcrossed in this case to family 35 instead of to D.

TABLE 8

Records of family 35 in two periods, of its crosses with the pure 4-toed stock D, of F_2 and of backcrosses of F_1 to family 35.

		PERCENTAGE			TOTAL NUMBER
		3-TOED	POOR 4-TOE	GOOD 4-TOE	
Family 35	Beltsville	68.9	26.2	4.9	1,976
	Chicago	57.9	35.9	6.2	356
F_1	35×D	12.0	24.0	64.0	25
	D×35	0.0	0.0	100.0	22
	Total	6.4	12.8	80.8	47
F_2	$F_1 \times F_1$	25.0	19.6	55.4	56
Backcross	F_1×35	31.5	30.1	38.4	73

There is an indication here of some matroclinous tendency in F_1 (exhibited equally by sons and daughters it may be added). Slight genetic differences between the parents from family 35 is most probable as an explanation, however, since as noted above there were demonstrable substrain differences in polydactyl tendency within the Beltsville stock. Various subdivisions of the F_2 and backcross data have been made but as nothing of significance was brought out, they are not given here.

Polydactyly is here more nearly dominant than recessive. There were 94 percent polydactyls in F_1 while F_2 showed a *smaller* percentage (75). This is the reverse of the situation in the crosses considered previously. It is suggested that dominance in the case of polydactyly (as in white spotting in guinea pigs) is a matter of character thresholds rather than of anything inherent in the genes themselves. The backcross of F_1 to family 35 gave intermediate percentages as might be expected.

MEANS AND STANDARD DEVIATIONS

In view of the great importance of non-genetic factors demonstrated in family 35, and in F_1(13×D) it is obviously futile to attempt to assign specific genes to different phenotypes. The most instructive course would

seem to be to compare the means and variabilities on the postulated scale of factor combinations. As the unit of measurement, it is convenient to take the distance between the threshold for any polydactyly and that for perfect development of the little toe. Approximately normal distributions are expected if variability is determined by independent factors of which none

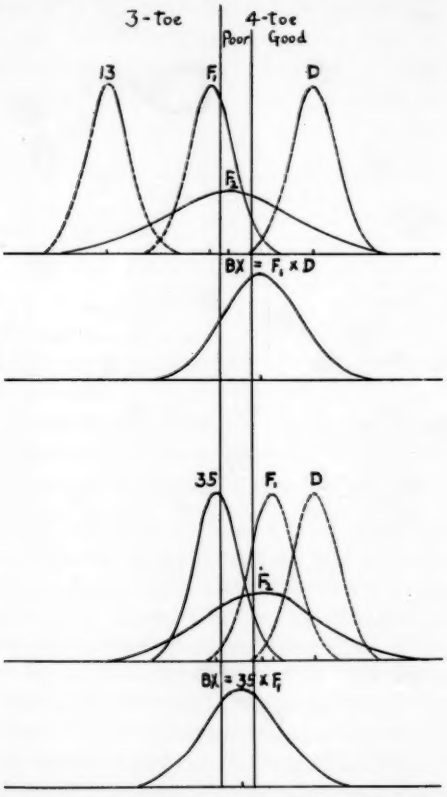

FIGURE 1.—Estimated distributions of strains 35 and D, and of F_1, F_2 and the backcross of F_1 to 35, relative to a scale on which the distribution curves are normal and the thresholds for any development of the little toe and for perfect development are separated by one unit (below). Estimated distributions of strains 13 and D, and of F_1, F_2 and the backcross of F_1 to D on the same scale (above).

are of major importance and whose effects combine additively (no dominance or epistasis). These assumptions will be shown to be at least in harmony with the data (figure 1).

Given a scale with two thresholds at a unit distance apart, a normal distribution is uniquely determined by the three percentages cut off by the

thresholds (in a population including all three types). To describe the distribution we find the inverse probability functions of the frequencies between each threshold and the median (that is, $\text{prf}^{-1}(q-.50)$ where q is the proportion below the threshold). These locate the thresholds relative to the mean of the population but on a scale on which the standard deviation is the unit of measurement. The reciprocal of the distance between the thresholds on this scale gives the standard deviation on the postulated scale on which the thresholds are separated by a unit distance. The thresholds can now be located by multiplying the previous expressions by the value for the standard deviation.

These calculations are shown below for the Beltsville and Chicago branches of family 35.

Family 35 Beltsville	*S.D.=1*	*S.D.=.861*
Threshold for good 4-toe, $\text{prf}^{-1}(.951-.500)=$	1.655	1.424
Threshold for poor 4-toe, $\text{prf}^{-1}(.689-.500)=$	.493	.424
Distance between thresholds	1.162	1.000
Family 35 Chicago	*S.D.=1*	*S.D.=.747*
Threshold for good 4-toe $\text{prf}^{-1}(.938-.500)=$	1.538	1.149
Threshold for poor 4-toe $\text{prf}^{-1}(.579-.500)=$	.199	.149
Distance between thresholds	1.339	1.000

No such construction is possible, of course, unless all 3 categories are present. The method is not satisfactory if the proportions in one of the extreme classes is small (unless the total number is very large) since a slight change in percentage makes a large difference in the inverse probability function in this region.

Fortunately the extensive data from family 35 make it possible to estimate the standard deviation in an inbred strain. This comes out .861 as indicated above for the Beltsville branch descended from one mating in the 12th generation. As already noted, the genetic variance was about 18 percent of the total. The standard deviation due to non-genetic factors can be obtained by multiplying by .90 $(=\sqrt{1.00-.18})$ giving .775. This is close to the estimate of the standard deviation (.747) in the Chicago stock, from one mating in the 22nd generation, in which analysis indicated no residual genetic variability.

The value .80 will be assumed as that characteristic of a genetically homogeneous stock and of the first cross between two such stocks. No high degree of refinement is of course possible.

F_1 from 35×D includes all three categories but the numbers are too small for a satisfactory calculation of the standard deviation from them. It is best to adopt .80 as the standard deviation, and locate the mean from

this and the proportion of *good* polydactyls. This puts the mean at 1.70 above the threshold for any polydactyly.

Strain D may also be assigned the same standard deviation as an inbred strain. It must be assigned a mean sufficiently above the threshold for perfect polydactyly that only a negligible portion of the frequencies fall below. This means at least 2.5 times the standard deviation or about 2.0 units above this threshold (3.0 units above the threshold for any polydactyly). Since the perfect polydactyly of strain D was reached by selection it is not likely that its mean is much higher. Thus we reach an estimate of $+1.70$ for the mean of F_1, intermediate between that of $-.42$ for family 35 and of $+3.0$ for strain D, all relative to the threshold for any polydactyly. An estimation from F_2 (which shows fairly high frequencies in all three categories (3-toe, poor 4-toe and good 4-toe) yields a mean of $+1.25$ (also intermediate) and a standard deviation of 1.86 which is very much larger than in family 35. The backcross data $(35\times D)\times 35$ give a mean at $+.62$ which is intermediate between F_1 and family 35. The standard deviation comes out 1.29, less than that of F_2 but greater than that of family 35, as should be the case if Mendelian factors are responsible for the genetic differences.

ESTIMATION OF NUMBER OF FACTORS

If α is the effect of a single gene and there are n such genes (with equal effect and no dominance or epistasis) the difference (Δ) between *extreme* plus and minus types is $2n\alpha$. The F_2 variance due to each pair of genes is $\frac{1}{2}\alpha^2$ and hence to n genes is $\frac{n\alpha^2}{2}$. The observed variance in F_2 is compounded of this genetic variance and the non-genetic variance which may be taken as measured by the variance of F_1 or of the parental strains (P). Thus $(\sigma_{F_2}^2-\sigma_P^2)$ gives the genetic variance of F_2. Eliminating α and solving for n gives $n=\frac{\Delta^2}{8(\sigma_{F_2}^2-\sigma_P^2)}$. In the backcross progeny the variance due to each pair of genes is $(1/4)\alpha^2$ so that the genetic variance should be just half as great as in F_2. A *minimum* estimate of the number of genes (ignoring chance variation) can be obtained from either of these formulae if Δ is taken as the difference between the parental strains (that is, these are assumed to be *extreme* plus and minus respectively). Any degree of dominance or of epistasis increases the estimate (WRIGHT in CASTLE 1921, SEREBROVSKY 1928, BERNSTEIN 1929).

Applied to the present, admittedly rather rough, data, the minimum number of factors differentiating strains 35 and D comes out *one* whether based on the variance of F_2 or that of the backcross data, or on Δ as the difference between 35 and D or as twice that between 35 and F_1. There is

therefore enough segregation to make it possible to assume that these strains are differentiated by only one major factor. It is also possible however that 35 has some plus factors not present in D and that the number of major factors is larger.

Figure 1 (lower half) shows a construction of the distribution of 35, D, F_1, F_2 and the backcross on the assumptions made above. The two thresholds divide the distributions of 35, F_2 and the backcross into the observed percentages while F_1 is assigned the same standard deviation as 35 and located so as to give the observed percentage below the threshold for perfect polydactyly. Strain D is also assigned the standard deviation of 35 and located so that no appreciable percentage falls below perfect polydactyly (mean at 2.0 above this threshold). It will be seen that with this construction, the variability of F_2 is shown as transgressing slightly both grandparental ranges. The backcross data are, however, in close accord with segregation of one factor.

Consider next the crosses involving family 13. In F_1 assume that the variability is wholly non-genetic (as indicated by the tests of F_1 3-toed and 4-toed), and that the resulting standard deviation is .80 as deduced for non-genetic variability from family 35. The mean can be located from the proportion of polydactyls and comes out at $-.56$ relative to the threshold for any polydactyly. The numbers and percentages in the 3 categories in F_2 are adequate for determination of mean and standard deviation. They yield a mean only a little higher than that reached for F_1, namely, $+.22$ but a much larger standard deviation, namely, 2.06.

In the upper part of figure 1, strain D is located as before, while strain 13 is assigned such a mean that F_1 is exactly intermediate between it and D. This locates 13 so far below the threshold that polydactyly is to be expected from it only as a rare anomaly (assuming the same standard deviation as in 35). The distribution for F_2 and the backcross are located strictly by the percentages of 3-toed, poor 4-toed and good 4-toed.

The mean of the backcross progeny ($+1.30$) falls about half way between the mean of F_1 ($-.56$) and that assigned to strain D ($+3.00$), giving a further check on the latter. The standard deviation of the backcross progeny (1.20) is greater than that of the inbred strain 35 but less than that of F_2.

In absolute values, the variance of $(13\times D)^2$ is greater than of $(35\times D)^2$ but the variance of the backcross $(13\times D)\times D$ is a little smaller than of $(35\times D)\times 35$. But calculation of the minimum number of factors in the case of 13 and D indicates 2 (using F_2 variance) or 4 (using backcross variance). There are certainly at least two major factors of comparable importance and 3 would be a better estimate.

The data from the crosses involving 32 and 2 are in such close agreement

that they may be combined for the present purpose. F_1 must be located sufficiently below the threshold for polydactyly that polydactyls are rare (1 in 26 from 32×D, none in 146 from 2×D). This indicates a mean in the neighborhood of −2.0. Calculation from the frequencies of the 3 categories in F_2 gives a closely similar mean (−1.74 from family 2 alone, −1.93 from

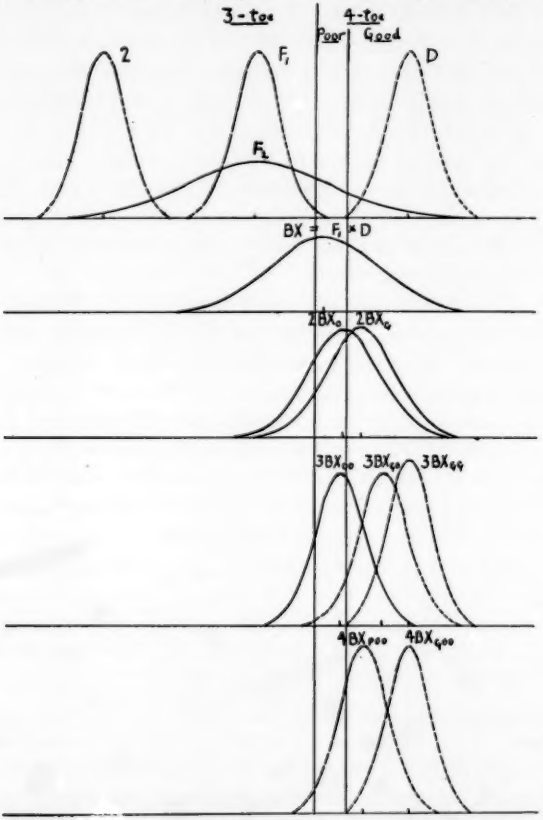

FIGURE 2.—Estimated distributions of strains 2 and D, and of F_1, F_2, and repeated backcrosses to strain D on the same scale as used in figure 1.

2 and 32 combined). The standard deviation of F_2 comes out 2.00 from strain 2 alone, 2.24 from 2 and 32 combined.

The backcross data yield a mean falling between the two thresholds (+.23 from 2 alone or combined with 32). This is about half way between the mean of F_1 (or of F_2) and that of D, as expected. The standard deviation of the backcross generation (1.70 from 2 alone, 1.69 from 2 and 32 combined) is between that of an inbred stock and of F_2.

The estimate of the minimum number of equal factors from the F_2 variance yields 4 or 3 depending on whether that of family 2 alone or that of 2 and 32 combined is used. The estimate from the backcross variance yields 3 in either case. It will be recalled that the analysis of the breeding tests of the backcross individuals indicated 3 as the least possible number of major factors and 4 as much more likely.

Figure 2 presents the results for these and later generations graphically. The close similarity of the results from the principal second backcross progenies is brought out. The progeny from 3-toed backcross individuals mated with D ($2BX_0$) comes at +.86 and that from the 4-toed backcross individuals, similarly tested ($2BX_G$) is at +1.46. The difference is only one-eighth of that between F_1 and D or one-fourth of that between the backcross group and D. On a one factor basis the difference should be the same as the latter. Again something like 4 equal factors are indicated. The standard deviations of both second backcross groups (1.15, 1.14) show a decrease from that of the first backcross (1.70).

In the 3rd backcross generation, repeated selection of 3-toed animals ($3BX_{00}$) has made it possible to keep the mean practically unchanged (+.79) but the standard deviation has fallen to practically the value of an inbred strain (.82). There is some indication here that the most important single gene has an effect amounting to from one-third to one-half of the difference between F_1 and D. This, however, is a maximum estimate.

These results are summarized in table 9.

TABLE 9

Estimated position of mean relative to threshold for polydactyly, and estimated standard deviation (S.D.), on scale on which threshold for perfect polydactyly is at +1.00. Standard deviation 0.80 in parenthesis assigned to inbred strains and first crosses on basis of results in strain 35.

	MEAN	S.D.		MEAN	S.D.
Strain D	+3.00	(0.80)	Strain 2	−7.00	(0.80)
Strain 35	−0.42	0.86	F_1, (2×D)	−2.00	0.80
F_1, (35×D)	+1.70	(0.80)	F_2, $(2\times D)^2$	−1.74	2.00
F_2, $(35\times D)^2$	+1.25	1.86	BX, ((2×D)×D)	+0.23	1.70
BX, ((35×D)×35)	+0.62	1.29	$2BX_0$, $(((2\times D)\times D)_0\times D)$	+0.86	1.15
Strain 13	−4.12	(0.80)	$2BX_G$, $(((2\times D)\times D)_G\times D)$	+1.46	1.14
F_1, (13 (D)	−0.56	(0.80)	$3BX_{00}$, $((((2\times D)\times D)_0\times D)_0\times D)$	+0.79	0.82
F_2, $(13\times D)^2$	+0.22	2.06	$3BX_{G0}$, $((((2\times D)\times D)_0\times D)_G\times D)$	+1.89	(0.80)
BX, ((13×D)×D)	+1.30	1.20	$3BX_{GG}$, $((((2\times D)\times D)_G\times D)_G\times D)$	+3.00	(0.80)

Extensive crosses have been made between families 2, 13, 32 and 35 (WRIGHT 1922b). These have been carried to F_2 on a large scale and to F_3 in many cases. No 4-toed young have appeared from matings involving only 2, 13 and 32, or in F_1 from matings involving 35. Among 81 F_2's from 13×35, there were 2 low grade polydactyls. It is thus probable that all of these strains have some factors in common in which they differ from strain D.

The results of experiments by CASTLE, STOCKARD and PICTET (discussed in the preceding paper of this series), readily fall in line with the above interpretation.

SUMMARY

The material used consisted of strains of guinea pigs which have been closely inbred since 1906. Three of these strains (2, 13, 32) bred true to the normal 3-toed condition of the hind feet. One of them (No. 35) produces about 31 percent 4-toed young. It has been shown previously that in this family the 3-toed and 4-toed animals of the same substrain have the same genetic constitution although different substrains differ genetically to a slight extent in percentage incidence. Strain D breeds true to perfect development of the little toe and hence is regularly 4-toed.

The crosses between 2 and D simulate one factor Mendelian heredity to a remarkable extent in the dominance of 3-toe in F_1, and apparent segregation in F_2 in a fairly close approach to a 3:1 ratio and in the backcross to strain D in a 1:1 ratio. This interpretation breaks down completely in the tests of the supposed segregants. These tests indicate that there are in reality at least 3 factors of comparable importance and more probably 4 by which strains 2 and D differ. There is a close approach to blending inheritance in a character which approaches alternative expression, because of physiological thresholds.

The crosses between strains 32 and D gave closely similar results to those of 2 and D, indicating genetic similarity of the normal strains 2 and 32.

The crosses between 13 and D, on the other hand, gave very different results. Many polydactyls appeared in F_1. These were shown not to differ genetically from the normals from the same matings. This result indicates that strain 13, though itself as normal as 2 and 32, is much closer to the threshold for polydactyly. The F_2 and backcross data show that 13 differs from D by at least 2 and more probably 3 major factors.

The crosses between 35 and D gave results compatible with the assumption of one differential major factor, complicated by minor factors.

In no case is there any indication of dominance apart from that due to transgression of physiological thresholds.

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UNISEXUAL MAIZE PLANTS AND THEIR BEARING ON SEX DIFFERENTIATION IN OTHER PLANTS AND IN ANIMALS

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Received November 23, 1933

Differences in the size or shape of the chromosomes of the two sexes have been noted in many dioecious plants as well as in animals. A compilation by SINOTO (1929) gives 7 species of bryophytes and 51 species of angiosperms so characterized. In all but one case the female gametes are alike. The male gametes are of two kinds. They either differ in size or shape of one pair of chromosomes, or one class of gametes has one or more chromosomes that the other class does not have.

In 33 dioecious species of bryophytes and angiosperms examined, no allosomes have been found. Animals also have species in which no inequality in chromosome construction is evident. WINGE (1922) does not find any visible difference in the chromosomes of Lebistes, although there is definite evidence that one chromosome pair is associated with sex differentiation. Many other animal species have been examined and no heteromorphic chromosomes found.

Nevertheless, WINGE (1923) states: "I have no doubt that sex chromosomes are found altogether throughout the whole of the vegetable kingdom in dioecious species, though it will not perhaps always be possible to demonstrate the same. . . . Unisexual individuals of either sex can in their autosomes be equipped with male and probably female tendencies, but the sex chromosomes are the normal regulating mechanism which as a rule determines the sex."

According to this view one particular pair of chromosomes carries the main sex-determining agency whether or not the members are visibly differentiated. It is obvious that differences in the amount of chromatin can not be the primary genetic agency in sex determination.

Unisexual plants, produced in controlled pedigree cultures, from a monoecious species showing equal chromosome pairs, give an illustration of a working mechanism that differentiates the two sexes. Dioecious maize has been developed and propagated through four generations. Two classes of sex-determining gametes are produced by the male plants; the female plants are monogametic.

GENES AFFECTING THE FLORAL EXPRESSION OF MAIZE

Many genes have a specific effect on the floral expression of maize. Some of the more noticeable ones are tassel seed, tunicate ear, anther ear, and several of the genes for dwarf plants.

It is our purpose to show that some of these have a definite tendency to further the development of female flowers and suppress male tendencies while others have a directly opposite effect. In other words, there are specific sex genes in maize.

Several genetic factors producing pistillate-flowered maize plants have been described. EMERSON (1920) has given his results with tassel ear and tassel seed. These genes later were renamed tassel seed-1 and tassel seed-2. The inheritance of tassel seed-4 has been given by PHIPPS (1928). These three factors are all recessive and when homozygous the terminal inflorescences have their staminate florets replaced by pistillate ones. There is some difference in the formation of the inflorescence characteristic for each gene but in general the result is the same. The usual panicle of maize is replaced by a mass of silks closely enfolded by the terminal leaves. The ovules are more or less unprotected by the glumes and as the inflorescence grows out from the surrounding leaves the developing seeds are exposed. In this condition they are easily infected by the smut fungus, and by the time the seeds are ripe, the whole inflorescence presents an unsightly object having no utilitarian value and apparently having no advantage for survival under natural conditions.

In tassel seed-1 the seeds are arranged on small spike-like structures that have some resemblance to the lateral inflorescences. Many of these small ears are grouped together with a densely matted arrangement of pistils as shown in figure 1. In tassel seed-2 (figure 2) the seeds are arranged in pairs. The whole structure resembles the normal staminate inflorescence much more closely than the other types. The seeds at the base of each branch are only partially covered by the glumes but the glumes become longer as they are placed farther out on the branches, and at the tips the ovules are often completely covered. Aborted anthers may be found with these seeds in varying numbers and stages of development. At the base they may be entirely rudimentary. They become more frequent and better developed at the tips of the branches. On some plants no anthers are found. On others they may all be aborted. On some the anthors may produce pollen in varying amounts up to the normal amount for the individual spikelet. This pollen from tassel seed plants is capable of fertilization.

Tassel seed-4 as described by PHIPPS differs from the other types in having the seeds more crowded and irregular and in having more than one pistil associated with each spikelet. Many of these supernumerary pistils are modified glumes. Anthers are also present with the ovules in the terminal inflorescences.

All of these tassel seed plants produce lateral inflorescences that are similar to the normal carpellate floral structure of maize. They differ in

having the seeds crowded into an irregular position due to the development of the second of the paired flowers that is usually aborted. Tassel seed plants in nearly every case can be identified from normal plants in this way even if the terminal inflorescence does not develop.

Three other tassel seed types have been named but their descriptions have not yet been published. They are dominant in their inheritance but otherwise are similar to the others just described. The dominant gene responsible for the tunicate ear of maize, when homozygous, also forces the development of ovules in the terminal inflorescences as described by COLLINS (1917) and shown here in figure 3.

FIGURE 1.—A terminal inflorescence of tassel seed-1 shortly after pollination.

Another group of genetic factors allows stamens to develop among the seeds of the lateral ear. Anther ear and several factors for dwarf plants fall in this class. Such anthers are usually poorly developed and are not permitted to shed pollen under natural conditions since they are enclosed by the husks. For a more complete discussion of genetic characters of this kind, see EMERSON (1924).

ENVIRONMENTAL INFLUENCES AFFECTING THE FLORAL EXPRESSION OF MAIZE

When grown in a shortened day, normal maize regularly produces seeds in the tassel as shown in figure 4. In such cases, often, no lateral inflores-

cences are formed. Both pistillate and staminate flowers are produced in varying amounts. The seeds produced in the tassels in this way give normal offspring when grown in a normal environment. The sporadic occurrence of seeds in the tassels on the main stalk and on the side branches of plants grown under natural field conditions, and the occurrence of tassel-like structures on the ears, are more difficult to account for. They are not readily associated with any external conditions since the usual fluctuations

FIGURE 2.—A terminal inflorescence of tassel seed-2 at the time of pollination, upper leaves removed.

of fertility and moisture do not consistently bring about these changes. They are not due to any simple combination of genetic factors since they do not reappear in later generations in the descendants of deviating plants much more than from normal plants. On the other hand, some strains of maize have a noticeable tendency to produce these abnormal flowers while other strains never do. This fact shows that some germinal combinations are more easily changed in this respect than are others. STRASBURGER, CORRENS, SCHAFFNER, YAMPOLSKY and others have given much evidence

showing the effect of external conditions upon the sexual expression of plants. RIDDLE, CREW, BANTA and others have done the same with animals. For the literature and an excellent discussion of this subject, the reader is referred to SHARP (1934). Since all other characters are modified in their expression by environmental influences, it is certainly to be expected that those complex characters associated with sex will also be modified by external conditions. But this variation does not preclude some

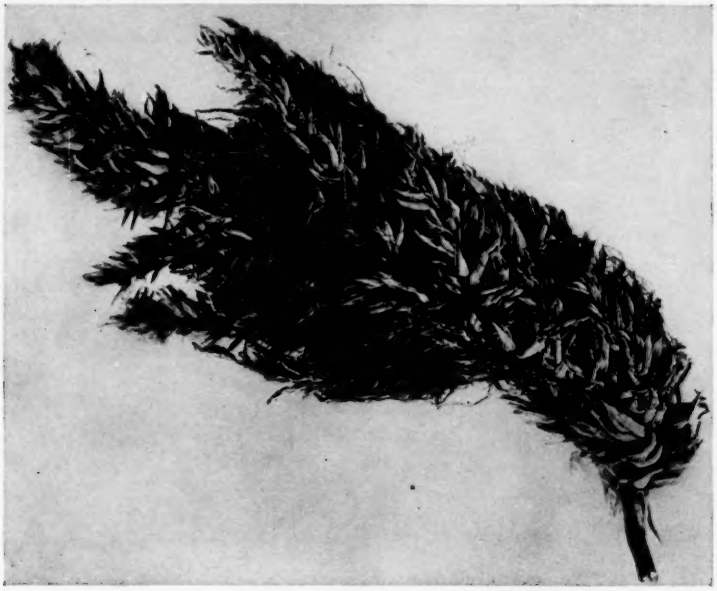

FIGURE 3.—Panicle of homozygous tunicate maize with mature seeds.

transmissible mechanism by which the numbers of two sexes are roughly apportioned.

All of the evidence shows that there is a clear-cut physiological gradient in maize from femaleness at the base of the plant towards maleness at the top. In both types of inflorescences this gradient is also operating from the base to the tip. In mixed inflorescences the female flowers are nearly always at the base and the male flowers at the tip. In related species of grasses, a similar situation exists. The genetic factors just described operate in some way to determine the points on this gradient where the development of the flowers begins. In such a complex of interacting forces their action is seldom complete but in general they determine the *balance*

of control such that the effect of one or the other allele directs development in one way or the other.

In studying the effect of genetic factors upon the floral expression of plants it is the usual practice to hold the environmental conditions as uniform as possible. In making such a study there is a natural tendency to overlook or minimize the importance of the external influences that may bring about similar results. For the same reason the physiologist uses ma-

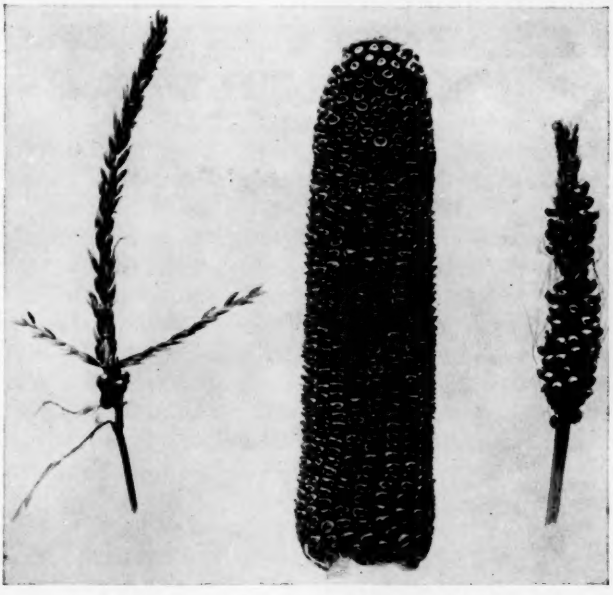

FIGURE 4.—Seeds produced in the terminal inflorescences of normal maize when grown in a shortened day, compared with a normal lateral ear (center) grown on the same kind of a plant in its usual environment.

terial that is germinally stable and varies the environmental conditions in many ways. He, likewise, has a tendency to overlook or minimize the importance of the germinal control of floral expression.

The normal monoecious maize plant is fundamentally a perfect-flowered organism, as shown by WEATHERWAX (1923). In the flowers of the terminal panicles the stamens are fully developed while the carpels are present only in a rudimentary condition. In the lateral spikes the reverse is the case. The stamens, ordinarily, do not develop beyond a primordial stage. The change from functionally carpellate to functionally staminate flowers is merely a suppression or stimulation of one or the other type. This change

may be brought about both by transmissable genes or by environmental influences. But under the usual conditions in which the plants are grown the flowers are relatively constant in their development. Otherwise maize would not be such an important crop plant.

Many unisexual plants and animals give evidence that they are basically bisexual and carry the potentiality to develop some or all of the opposite sex characters. Sex reversal is simply a process of stimulation of certain potentialities that are usually dormant and of suppression of other characters normally functioning.

ORIGIN OF DIOECIOUS MAIZE

The pistillate-flowered maize plants described above are wholly or largely female in function. Several other genes are known that sterilize entirely the pistillate flowers. One of these is silkless, a gene that causes the ovaries to abort. The lateral inflorescences are otherwise normal in development, but the spike, enclosed in its usual protective husks, is entirely barren. Stalks wholly without lateral flowers are associated with at least two other genes. Both of these types lacking pistillate flowers have well-developed stamens in the terminal inflorescences and are entirely male in function.

In 1925 the production of dioecious plants in a species that had previously never shown this condition was attempted by crossing a functionally pistillate individual with a functionally staminate one. For this purpose a silkless plant was used as the male and tassel seed-2 as the female. The behavior of the first and later generations of this cross have been described (JONES 1931, 1932). Briefly, the first generation was entirely normal with both types of flowers in their usual position. In later generations double recessives of tassel seed-2 and silkless were found to be indistinguishable from tassel seed-2 by itself. Apparently this tassel seed gene has the ability to nullify the action of the silkless gene. Plants having the composition *sk sk* ts_2 ts_2 produce seeds both in the lateral as well as the terminal flowers and are female in function. When such plants are crossed with silkless individuals heterozygous for tassel seed, *sk sk* Ts_2 ts_2, and male in function, the result is a progeny made up of the same two classes as the parents.

In this way the dioecious condition propagates itself. Fourteen families in all have been grown. Eight of these were carried two generations and six were continued to the fourth generation. The results with the fourteen families are given in table 1. In a total of 967 plants, 390 are male and 515 female, with 61 monoecious hermaphrodites.

GENETIC COMPOSITION OF THE DIOECIOUS PLANTS

The evidence that the female plants are homozygous for both tassel seed and silkless is derived from several sources. In the first place, plants

heterozygous for both factors when selfed give normal, silkless and tassel seed plants, in a ratio of 9:3:4. This is as expected on the assumption that the recessive silkless gene has no effect when associated with homozygous recessive tassel seed. The actual count is 36 normal, 17 silkless and 30 tassel seed with 14 individuals having normal tassels but no lateral inflorescences. Such plants can not be classified either as silkless or normal but may be either. Dividing them in the same ratio as the same two types found and adding to the others, a ratio of 46:21:30 is obtained where expectation is 54:18:24.

If the female plants are homozygous for seed sterility, even though producing good seeds, when crossed by recessive silkless with normal tassels, they should give all silkless plants. Five progenies have been grown totaling over 100 individuals and all but one have been silkless. This one could easily be an out-cross.

Some tassel seed plants produce a small amount of pollen. Such pollen used on female plants gave a progeny entirely made up of tassel seed plants.

With the evidence from these three sources it is proved that the female plants are *sk sk* ts_2 ts_2 in composition and are monogametic. The male plants are *sk sk* Ts_2 ts_2 in composition and are digametic. Male and female plants in the third generation of dioecious propagation are shown in figure 5. Representative terminal and lateral inflorescences of a female plant and lateral sterile inflorescences only of a male plant are shown in figure 6.

The growth and arrangement of the terminal flowers of tassel seed are such that it is difficult to guard against undesired pollen in controlled pollinations. For this reason the lateral spikes are generally used for pollination. In many cases the female plants make no lateral inflorescences necessitating the use of terminal flowers. Even the lateral flowers can not be completely protected. In every case in which pollen from other kinds of corn gains access, the result is a normal monoecious hermaphrodite. Moreover, outside pollen also makes possible female-appearing plants heterozygous for silkless (ts_2 ts_2 *Sk sk*) instead of the double homozygotes. These plants can not be detected and when used as females give hermaphrodites, females and males in the ratio of 1:2:1. If we take all progenies in table 1 with two or more hermaphrodites, we have a count of 34 hermaphrodites: 87 females: 45 males—a fair approximation of a 1:2:1 ratio.

Twenty-one progenies produced only male and female plants. Twenty-one more showed only one hermaphrodite. In many cases, following generations gave no hermaphrodites, showing that these unexpected individuals could have been out-crosses. In the remaining 12 progenies out of a total of 54, there were two or more hermaphrodites. It seems unreasonable to

attribute all of these to accidental out-crossing and to parents heterozygous for silkless, especially in view of the fluctuating variability of the female plants themselves, although these two possibilities undoubtedly account for many of the off-type plants.

DEVIATION IN SEX RATIO

The 21 progenies with no hermaphrodites produced 170 males and 210 females, a ratio of 1 to 1.24 or 44.7 percent of males. A deficiency of males

FIGURE 5.—Dioecious maize: female (left) with mature seeds in both terminal and lateral inflorescences; male (right) with pollen-bearing panicle and sterile lateral inflorescence.

has been noted in many dioecious plants, particularly by CORRENS (1928) in Melandrium and Rumex, and WINGE (1923) in Cannabis and Humulus. In these plants WINGE attributes the differences to unequal fertilizing ability of the male-determining pollen grains with their smaller chromatin content. He cites NOHARA's (1923) results with Spinacia showing equality in the number of both sexes in a species where no chromosome differences have been found. A difference in chromosome number or size is apparently not the deviating factor in this material. There is no reason evident why

the female plants with two homozygous recessive factors and their associated chromosomes should have a higher survival value than male plants with only one such pair.

VARIATION IN PISTILLATE PLANTS

It is a common observation that inbred strains of maize vary markedly in the relative development of their pistillate and staminate flowers. Some strains are good pollen producers and are less well-developed in seed formation. Others give good grain yields when properly pollinated by other

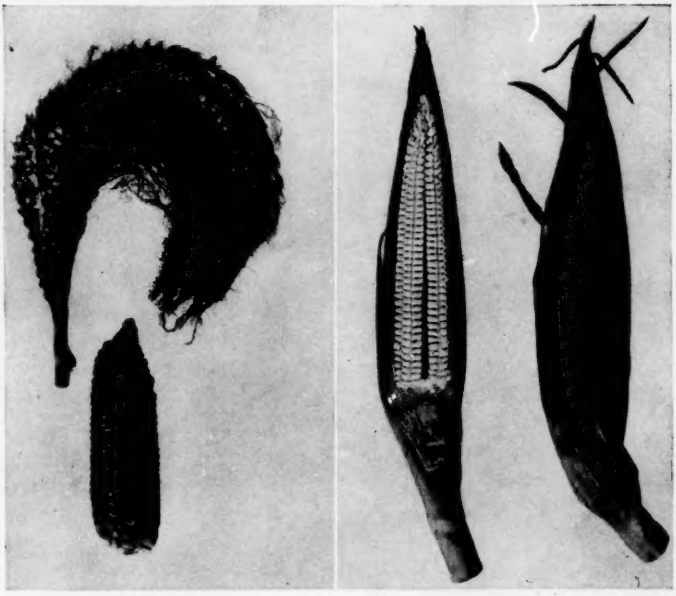

FIGURE 6.—Dioecious maize: mature seeds in the terminal and lateral inflorescences of a female plant (left) and sterile lateral inflorescences of a male plant (right).

plants but shed very little pollen themselves. Other strains are about equally well-developed.

Tassel seed types also vary in the amount of seed borne in the terminal panicle and in the amount of pollen produced. In the original tassel seed-2 plants obtained from Dr. R. A. EMERSON, no anthers were formed A representative terminal inflorescence is shown in figure 2. After crossing with normally tasseled plants for several generations during the process of establishing the dioecious condition and then for four generations afterwards a tendency to form anthers has been strongly developed in all fami-

TABLE 1

The progeny of dioecious maize families in successive generations.

FAMILY NUMBER	YEAR GROWN	CLASSIFICATION OF PROGENY		
		♂	♀	☿
1	1930	11	7	0
	1931	9	14	0
	1932	11	6	1
	1932	12	9	0
	1933	1	15	0
Total		44	51	1
2	1930	5	6	0
	1931	7	4	1
Total		12	10	1
3	1930	5	12	0
	1931	5	4	0
	1932	8	12	0
	1932	10	8	1
	1933	8	3	1
	1933	7	11	0
	1933	9	6	0
	1933	5	8	1
Total		57	64	3
4	1930	6	14	0
	1931	6	11	1
	1932	12	9	0
	1933	4	12	0
Total		28	46	1
5	1930	10	10	0
	1931	11	10	1
	1932	10	8	2
	1932	10	12	0
	1933	11	5	0
	1933	5	9	3
Total		57	54	6
6	1930	12	8	1
	1931	6	13	2
	1932	8	13	0
	1932	0	9	5
	1932	9	7	1
	1932	18	5	0
	1933	7	8	1
	1933	5	12	2
	1933	2	8	6
	1933	7	12	0
	1933	5	8	0
Total		79	103	18
7	1930	9	6	1
	1931	8	9	1
Total		17	15	2

TABLE 1 (*Continued*)

8	1930	9	10	1
	1931	7	14	0
Total		16	24	1
9	1930	8	4	1
	1931	9	8	1
Total		17	12	2
10	1930	4	14	2
	1931	6	15	1
	1932	7	14	1
	1932	6	7	6
	1933	2	14	1
	1933	7	7	6
	1933	4	10	1
Total		36	81	18
11	1930	3	17	1
12	1930	8	10	2
13	1930	5	13	1
14	1930	6	10	2
	1931	5	5	2
Total		11	15	4
Total of all families		390	515	61

lies, indicating an accumulation of germinal modifying factors, in spite of the fact that good female plants have been selected as progenitors in each generation. Figure 7 shows eight stages of variation from nearly complete female to nearly complete male, represented by plants in the fourth generation of dioecious propagation.

In the first stage all of the flowers have well-developed ovaries and pistils. There are a few rudimentary anthers in the terminal flowers of the main spike and at the tips of some of the branches. The whole inflorescence is curved and drooping due to the weight of the developing seed. In the intermediate stages there are no pistils in the flowers at the tips of the main spikes. The flowers with functioning ovaries become fewer in number and those with anthers more and more prominent. The panicles become more upright and spreading. Finally stages are found in which very few seeds are produced and in some the pistillate flowers form no seeds. Their presence is noted by the few scattering pistils.

Some of the hermaphrodites found in the dioecious progenies may be females modified to a point where no silks are visible in the tassels. Such plants would still have lateral ears with irregular rows. No such plants have been noted although not all of the hermaphrodites were examined with this point in mind.

Many of these stages of variation are found within the same progeny or in the same family in different generations. There are, however, persistent differences in different families with respect to the stage of sex integradation indicating genetic control in some way. These differences can not be easily shown statistically.

VARIATION IN STAMINATE PLANTS

The silkless gene was first found in an inbred strain of yellow flint (JONES 1925). A similar gene, found by Dr. E. G. ANDERSON in other material, proved to be the same when tested. Silkless has usually been quite clear cut in its expression. A few seeds have been found in the lateral inflorescences

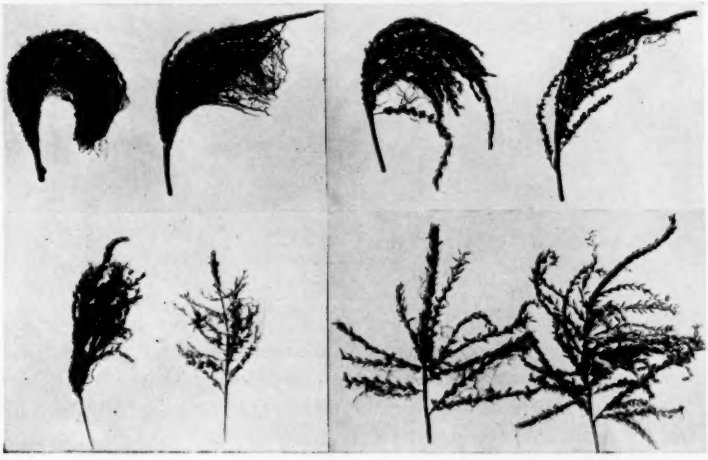

FIGURE 7.—Mature panicles from female plants of dioecious maize in the fourth generation showing variation in the number of pistillate and staminate flowers.

of several male plants. Some male plants in the fourth generation in one dioecious family produced a few short pistils on two ears that were otherwise barren. Pollen was applied to these but no seeds developed. A sterile structure of this kind, with one short pistil, is shown in figure 8 together with other cobs showing variation in the number of anthers produced. In many silkless families anthers are formed at the tips of nearly all of the barren spikes. These anthers are poorly developed and rarely contain pollen.

SPECIFIC SEX-DETERMINING GENES

In this dioecious maize there are two specific sex-influencing genes whose locations are known. One of these is kept in the heterozygous con-

dition in one sex, the other homozygous in both sexes. The interaction of both is necessary to maintain the unisexual condition. A clear presentation of a similar case has been made by ALLEN (1932).

So far the chromosome formation of this material is entirely normal. No visible differences in the male and female-determining chromosomes can be seen. The enforced heterozygosity maintained in one sex makes it possible for chromosome irregularities such as deficiencies or inversions to persist if they should occur and are viable. There is also the probability that recessive genes will accumulate in the protected chromosome in the male and this in time may have an effect on the sex expression.

In this material the male is the heterozygous sex. By using tassel seed

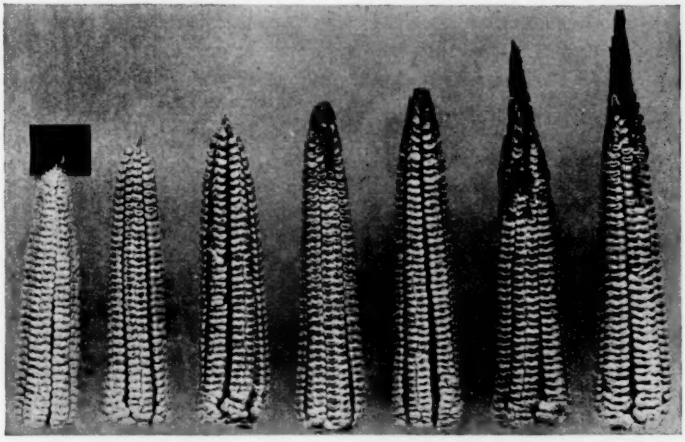

FIGURE 8.—Sterile lateral inflorescences from male plants in the fourth generation showing variation in the number of pistils and anthers.

genes with the gene for barren stalk, EMERSON (1932) has developed two types of dioecious maize. In one of these the male is the heterozygous sex, in the other the female is the heterozygous sex. The latter has been made by using one of the dominant tassel seed genes instead of a recessive.

No male plants have been found with ovules in the tassels or on the terminal flowers of side branches where they occur infrequently on normal plants. On the other hand there is a marked tendency for these individuals to produce anthers in the lateral spikes even though heterozygous for the tassel seed gene. The terminal flowers are well-developed and produce an abundance of pollen. This is due in part to their nutritional advantage in being carried on plants that do not have the burden of producing seeds. Nevertheless the silkless gene seems to be a specifically male-promoting

gene, suppressing female tendencies. The tassel seed gene is distinctly a female-promoting gene. The staminate flowers in the panicle are suppressed and the carpellate flowers that have long been dormant are forced into development. The secondary flowers in the spikelet of the lateral inflorescences are also forced into development as shown by the irregular arrangement of the kernels and their small and crowded condition. And in addition, the sterilizing effect of the silkless gene is entirely suppressed when both are acting together.

RELATION OF DIOECIOUS MAIZE TO SEX IN OTHER ORGANISMS

Although we should not expect to find any close agreement between this sex mechanism in maize, that has come into operation almost overnight, with processes that have been developing in other species through geological epochs, there are certain features in common. In its important characteristics this situation in maize is closely in line with the theories of sex-determination advanced by CORRENS and GOLDSCHMIDT and supported by MULLER and PAINTER. One specific gene holds the *balance of control* over the sex of the offspring. In its action this gene is wholly dependent upon the support of at least one other gene located in a different chromosome. Other genes working with physiological modifiers, both internal and external, influence the development of the sex organs and bring about varying degrees of intergradation between the two sexes. None of these factors has been specifically located but definite evidence for their existence is at hand. They are complex in their action and widely distributed in the chromosomes.

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SELF FLOWER-COLOR INHERITANCE AND MUTATION IN MIRABILIS JALAPA L.

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Received December 14, 1933

Mirabilis jalapa, commonly known as "Four O'clock," "Sweet Marvel of Peru," or "Printoponite," belongs to the Nyctaginaceae. This species is cultivated for the brilliant color and pleasing odor of its flowers. Due to its simplicity, the breeding behavior of the self flower colors has furnished classical material for illustrating the simple laws of inheritance. It may be shown, however, that the breeding behavior of these self flower colors is not so simple as was first implied, and that where formerly there were only a few flower-color classes recognized, now there are many. CORRENS (1902, 1904), the first to investigate this species genetically, failed in many cases to recognize and classify the flower colors correctly. His attention was chiefly directed toward an interpretation of the "yellow×white" varietal cross in which reds (red being used in a very general sense to cover a number of different shades) were obtained in the F_1 and F_2, but he failed to distinguish between the F_2 red flower-color types. The careful distinction between the color classes came with the work of MARRYAT (1909). Aided by the discovery of "recessive white" (MARRYAT'S W7), she was able to detect the genotypes of all the material used in her investigations. The "rose pink" homozygote and "light pink" heterozygote of KIERNAN and WHITE (1926) were probably the first true pinks known to genetic literature on this species. The pink so frequently referred to in text-book illustrations as resulting from a "red×white" four o'clock cross is probably not pink, but magenta (see also MARRYAT 1909, and KIERNAN and WHITE 1926).

M. jalapa was introduced into Europe by the Spaniards in 1596. It is native to Peru, as one of its common names, "Sweet Marvel of Peru," suggests. HEIMERL (1901) found it to be native also to northern Mexico and the southern boundary of the United States. Under cultivation color varieties not known to the wild condition have occurred, and are recurring, spontaneously.

MATERIALS AND METHODS

This investigation (1) reviews all crosses, and reciprocals, previously made with true-breeding self flower-color varieties of *M. jalapa;* (2) attempts to describe the breeding behavior of Flesh Pink and Light Rosaline Purple, two new true-breeding types which have been found during the

progress of the investigation, and reports the occurrence of Violet-Purple and Light Buff; and (3) attempts to throw light on the nature of mutation in flower color in this species.

In many respects *M. jalapa* is desirable material for crossing work. Its pollen grains are large (about 189μ in diameter) and can be administered singly to the stigma if desired. It is self-fertilized, its flowering season is long, it sets seed profusely, any particular plant can be carried over from one year to another by roots, and the characters in general are clear cut. However, in many cases, the flower-color heterozygotes cannot be distinguished at sight, as was formerly thought. Undesirable characteristics may be mentioned, such as the setting of only one seed per flower, the opening of the flowers for only a short time each day, and the susceptibility to cross-pollination by bees and nocturnal moths. In order to insure self- or cross-pollination, as the case may be, frames covered with cheese cloth have been used satisfactorily. The mature buds anthese about 6 P.M. in July (at The Blandy Experimental Farm), or about one half hour before sunset, and remain open until shortly after sunrise the next morning. Depending on temperature conditions this is variable, as is also the time of dehiscence of the anthers. The flowers with the darker hues open first. In crossing it is necessary to emasculate the mature buds of the female parent about three hours before time of anthesis, thus eliminating the possibility of self-pollination by precocious dehiscence of its anthers. In unemasculated flowers the style and anther filaments coil about each other some hours after anthesis, and if cross-pollination has not already taken place, self-fertilization will occur from self-pollen placed upon the stigma by this coiling.

The true-breeding self-flower color varieties of *M. jalapa* known at the present are Crimson, Yellow, Dominant White, Recessive White, Rose Pink, Flesh Pink, and Light Rosaline Purple. To this list probably will be added Violet-Purple and Light Buff. These last two have not been studied sufficiently to ascertain their breeding behavior (they were only discovered in 1933). Light Rosaline Purple has been used in only a few crosses. The known homozygotes are given in table 2. Similarly in table 3 the heterozygotes are given, with their corresponding genotypes.

The names of the homozygotes and heterozygotes describe as accurately as possible the colors of the flowers, and are given on the basis of comparisons with Ridgway's Color Standards and Nomenclature (1912). MARRYAT (1909) has plates of Crimson, Magenta, Magenta Rose, Yellow, White, et cetera, classes. The calyx makes up the showy part of the flower, having a tubular throat from 4 to 5 cm in length, and a rotate margin from 3.5 to 4.5 cm across, the latter being the highly colored portion. The throat color, inside and out, plus a star-shaped region on the upper surface run-

ning from the throat to the margin, is less intensely colored than the margin (see text figure 1). In "red" flowers the throat color is dull magenta; in "yellows," greenish yellow; in "whites," greenish white; and in "pinks," greenish pink. The style and anther filaments are generally about the same color as the throat of the flower, but deepen in intensity toward the extremities. The anthers (5 in number) and stigma correspond in color to the throat, except that they are more intensely colored. The stalk internodes, and particularly the nodes, are always somewhat magenta-colored in plants with "red" flowers; but in all others the stalks are entirely green.

On the basis of related genotypes and flower colors there are nine groups of heterozygotes (table 3). It is obvious from a study of this table that in many cases, types within a group cannot readily be distinguished at sight.

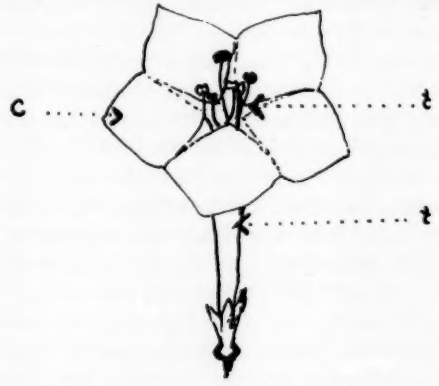

FIGURE 1.—Diagram of the color regions of the *M. jalapa* flower. c—region of intense color, t—color of the throat, less intense.

Depending on environmental conditions, such as sunlight, soil, et cetera, two plants of the same genotype may vary in intensity of color. A plant in the early flowering stages generally bears flowers which are a shade darker than those borne at a later stage. Due to this fluctuation in color, Yellow and Pale Yellow, Rose Pink and Light Pink, Light Rosaline Purple and Pale Rosaline Purple, homozygotes and heterozygotes respectively, may not be distinguishable. Naturally it is impossible to distinguish between the different genotypes of Crimson, Yellow and White, except by breeding.

Stocks of *M. jalapa* used in this investigation were grown from seed obtained from the following sources:—

(1) BROOKLYN BOTANIC GARDEN of the same lines used in the investigations of KIERNAN and WHITE (1926); and

(2) STARK BROTHERS, Seedsmen and Nurserymen, Louisiana, Missouri (Original Burbank stock).

FLOWER-COLOR INHERITANCE

At this point it is essential that a brief summary be made of the mechanism of flower-color inheritance. Crimson, Yellow, and Dominant White are the most common of the true-breeding varieties. In Yellow, there is a soluble yellow pigment base. ONSLOW (1925) thinks that all the varieties of *M. jalapa* "sprang originally from the crimson, which apparently contains a mixture of magenta anthocyanin and soluble yellow pigment." By the loss of anthocyanin the yellow variety was obtained, and by the loss of yellow pigment, the white varieties resulted.

The whole range of flower-color classes (see tables 2 and 3) of this species has been interpreted as being due to the action and interaction of factors at two loci (CORRENS 1902, 1904, MARRYAT 1909, KIERNAN and WHITE 1926). The one controls the color base, the other modifies the color base. Beginning with the work of KIERNAN and WHITE (1926) it may be shown that the latter locus is typified by an allelic series. The loci may be designated as the *Y* and *R* loci, respectively, using the terminology of KIERNAN and WHITE (1) with additional symbols to indicate the other factors of the *R* series (2).

Symbols:—

(1) *Y*—factor for yellow color, or color base;

y—allele of *Y*, absence of color base;

R—factor which modifies *Y* to red, and in the absence of *Y*, white color results;

Rp—allele of *R*, modifies *Y* to red, and when *Y* is replaced by *y*, it gives rose pink color;

r—allele of *R* and *Rp*, gives non-modification with *Y*, and in its absence (in the presence of *y*) white color results;

(2) *rp*—allele of the *R* series, gives non-modification with *Y*, and when *Y* is replaced by *y*, flesh pink color results. *rp* is dominant to *r*;

Rpl—factor for light rosaline purple, also allele of the *R* series. It is incompletely dominant to *R* and *Rp*, and presumably dominant to *r* and *rp*. In the presence of *Y* it produces red color. A complete account of its breeding behavior cannot be given at present.

For clearness the several crosses made during this investigation will be treated individually. Reciprocal crosses give the same results and are incorporated in table 1. From all the data obtained by myself, as well as those of previous experimenters, it is evident that the factors *Y* and *R* are inherited independently of each other.

(1) Crimson×Yellow. Three genotypes of Crimson have been identified in this investigation (see table 2-1, 2 and 3-1), which were postulated by KIERNAN and WHITE (1926). Those with genotypes *YYRR* and

YYRpRp breed true, while the third, *YYRRp*, upon selfing gives the first, second, and third types in the ratio of 1:1:2. On different occasions I have used the first and second types in this cross. Corresponding to the Crimson genotypes there are three of Yellow, *YYrr*, *YYrprp*, and *YYrrp* (see table 2-3, 4 and 3-2). The first and second breed true, while the third gives again the three types in an approximate 1:1:2 ratio. The F_1 heterozygote of this cross is Orange Red, Scarlet Red, or a shade intermediate to these colors, depending upon the parental genotypes. In the presence of *R*, orange red color is expected, and in the presence of *Rp*, scarlet red (see table 3-5, 6, 7, 8). The crosses from which the data are given (see table 1-1a-1b) had as the Crimson parents *YYRR*, and *YYRpRp* types, respectively. In the F_2 progeny, Crimson, Orange Red (or Scarlet Red), and Yellow, are obtained in an approximate 1:2:1 ratio.

(2) Crimson×Dominant White. Magenta is the only F_1 heterozygote which has been reported for this cross. By appropriate crosses, using Crimson and Dominant White, of the *YYRpRp* and *yyRR* types, respectively, Rhodamine Purple has been obtained (see table 1-2b). This heterozygote may appear indistinguishable from that of the Crimson×Rose Pink cross, but genotypically it is different, and theoretically less intense in color. By still further varying the cross, using a Crimson parent of the *YYRRp* type, two color heterozygotes should be obtained, Magenta and Rhodamine Purple. This cross has been made, but the results are not as satisfactory as theoretically they should be. Due to the similarity of these colors, it is extremely difficult to identify them in a population, even with the aid of a color chart. In the F_2, Crimson, Magenta (or Rhodamine Purple), and White (in the presence of the factor *Rp* in the homozygous condition, 1 White: 2 Light Pink: 1 Rose Pink, would be expected instead of all White) are obtained in an approximate 1:2:1 ratio.

(3) Crimson×Rose Pink. The original of this cross was not satisfactory, as pointed out by KIERNAN and WHITE (1926). But the postulation that Crimson, Rhodamine Purple, and Rose Pink would be obtained in an approximate 1:2:1 ratio in the F_2 has been verified in my work (see table 1-3). The F_1 heterozygote may be of the *YyRRp* or *YyRpRp* type (see table 3-14, 15), depending upon the genotype of the Crimson parent. I have not been successful in distinguishing between plants differing in this respect.

(4) Crimson×Flesh Pink. The Crimson of this cross was of the genotype, *YYRpRp*, and the Flesh Pink of the *yyrprp* type. The Rose Red of the F_1 is indistinguishable from that of the Yellow×Rose Pink cross, though theoretically it is of a deeper shade. It is of the genotype *YyRprp* (see table 3-12). The F_2 population is distributed in the approximate ratio of 1 Crimson:1 Yellow:3 Rose Pink:1 Flesh Pink:2 Scarlet Red:2 Rhoda-

TABLE 1

Data from crosses of flower-color varieties of M. jalapa.

CROSSES																		
PARENT GENOTYPE × PARENT GENOTYPE	F_2 COLORS	CRIMSON	YELLOW	WHITE	ROSE PINK	FLESH PINK	L. ROS. PURPL.	ORANGE RED	SCARLET RED	MAGENTA	RHOD. PURPLE	MAGENTA ROSE	ROSE RED	LIGHT PINK	PALE YELLOW	P. ROS. PURPL.	TOTALS
1a. Crimson *YYRR* × Yellow *YYrr*	ob.	40	42					87*									169
	ex.	42.2	42.2				84.5										
1b. Crimson *YYRpRp* × Yellow *YYrr*	ob.	26	31						65*								122
	ex.	30.5	30.5						61.0								
2a. Crimson *YYRR* × Dom. White *yyRR*	ob.	24		30						65*							119
	ex.	29.7		29.7						59.5							
2b. Crimson *YYRpRp* × Dom. White *yyRR*	ob.	13		17							52*						82
	ex.	20.5		20.5							41.0						
3. Crimson *YYRR* × Ro. Pink *yyRpRp*	ob.	32			42						73*						147
	ex.	36.7			36.7						73.5						
4. Crimson *YYRpRp* × Fl. Pink *yyrprp*	ob.	9	10		19	7			16		20		28*		17		126
	ex.	7.8	7.8		23.6	7.8			15.7		15.7		31.4		15.7		
5. Crimson *YYRpRp* × Rec. White *yyrr*	ob.	5	2	7	6				11		13		21*	16	14		95
	ex.	5.9	5.9	5.9	5.9				11.8		11.8		23.7	11.8	11.8		
6. Yellow *YYrr* × Dom. White *yyRR*	ob.	12	13	50				26		22		55*			26		204
	ex.	12.7	12.7	51				25.5		25.5		51.0			25.5		
7. Yellow *YYrprp* × Ro. Pink *yyRpRp*	ob.	20	24		20	24			41		40		67*	25	42		303
	ex.	18.9	18.9		18.9	18.9			37.8		37.8		75.7	37.8	37.8		
8. Yellow *YYrprp* × Fl. Pink *yyrprp*	ob.		24			32									36*		92
	ex.		23.0			23.0									46.0		
9. Yellow *YYrr* × Rec. White *yyrr*	ob.		13	9											24*		46
	ex.		11.5	11.5											23.0		
10. Dom. White *yyRR* × Ro. Pink *yyRpRp*	ob.			29	22									48*			99
	ex.			24.7	24.7									49.5			
11. Dom. White *yyRR* × Fl. Pink *yyrprp*	ob.			2		4								7*			13
	ex.			3.2		3.2								6.5			
12. Dom. White *yyRR* × Rec. White *yyrr*	ob.			17*													17
	ex.			17													
13. Ro. Pink *yyRpRp* × Fl. Pink *yyrprp*	ob.				53*	20											73
	ex.				54.6	18.2											
14. Ro. Pink *yyRpRp* × Rec. White *yyrr*	ob.			12	8									26*			46
	ex.			11.5	11.5									23.0			
15. Fl. Pink *yyrprp* × Rec. White *yyrr*	ob.			7		27*											34
	ex.			8.5		25.5											
16. L. Ros. Pur. *yyRplRpl* × Dom. White *yyRR*	ob.			4			3									6*	13
	ex.			3.2			3.2									6.5	

* F_1 flower color of respective crosses.

mine Purple:2 Pale Yellow:4 Rose Red. No Whites were obtained in my results (see table 1–4), which would have been expected had the Crimson parent been of the *YYRR* type.

TABLE 2

Known flower-color homozygotes of M. jalapa. Light Rosaline Purple is excepted since its genotypic formula is uncertain.

(1) *YYRR* Crimson	(3) *YYrr* Yellow
(2) *YYRpRp* Crimson	(4) *YYrprp* Yellow
(5) *yyRR* Dominant White	(7) *yyRpRp* Rose Pink
(6) *yyrr* Recessive White	(8) *yyrprp* Flesh Pink

TABLE 3

Groups representing related genotypes of flower-color heterozygotes of M. jalapa.

(1) *YYRRp* Crimson (group 1)	(2) *YYrrp* Yellow (group 2)
(3) *yyRr* White (group 3)	(4) *yyrrp* Flesh Pink (group 4)
(5) *YYRr*	(9) *YyRr*
(6) *YYRpr* Orange Red to Scarlet Red	(10) *YyRpr* Magenta Rose to Rose Red (group 6)
(7) *YYRrp* (group 5)	(11) *YyRrp*
(8) *YYRprp*	(12) *YyRprp*
(13) *YyRR*	(16) *Yyrr*
(14) *YyRRp* Magenta to Rhodam. Purp.	(17) *Yyrrp* Pale Yellow (group 8)
(15) *YyRpRp* (group 7)	(18) *Yyrprp*
(19) *yyRRp*	
(20) *yyRpr* Light Pink to Rose Pink (group 9)	
(21) *yyRrp*	
(22) *yyRprp*	

(5) Crimson × Recessive White. Difficulty was encountered in obtaining the Recessive White for this cross (see table 2–6). This type was originally discovered by MARRYAT (1909), and is distinct from the Dominant White. In the F_1 of this cross MARRYAT obtained Magenta Rose plants, indistinguishable from that of the Yellow × Dominant White cross. In my experience I have obtained Rose Red (see table 3–10) in the F_1, due to the *Rp* factor of the Crimson parent, not present in MARRYAT's experiments. In the F_2 approximately 1 Crimson:1 Yellow:1 White:1 Rose Pink:2 Orange Red to Scarlet Red:2 Rhodamine Purple:2 Light Pink:2 Pale Yellow:4 Rose Red occurred (see table 1–5). Had factor *Rp* not been present in the Crimson parent, more Whites would have been expected.

(6) Yellow×Dominant White. Magenta Rose was obtained in the F_1 (see table 3–9), the parental genotypes being *YYrr* and *yyRR*, respectively. No "flaking" occurred in a total of 19 plants. "Flaking" is the term used by MARRYAT (1909) to designate bi- and tri-colored flowers in *M. jalapa;* for example, flowers marked with crimson and white, yellow and white, or crimson, yellow and white, et cetera. The color markings in bi- and tri-colored forms vary, ranging from a few small dots or striations to an area half or three-quarters of the flower. According to MARRYAT "it is characteristic of Mirabilis that bi- and tri-coloured individuals almost always produce a few whole-coloured flowers; *e.g.* a plant having most of its flowers white flaked with yellow, bears some pure yellow and some pure white blossoms, and so on.") MARRYAT (1909) found flaking in all her F_1 plants of this cross and thought it was peculiar to the particular cross, whereas in my experience it is due rather to the material. Her material must not have been homozygous for self-flower color. My results of the F_2 population of this cross (see table 1–6) show a close dihybrid ratio of 1 Crimson:1 Yellow:4 White:2 Orange Red:2 Magenta:2 Pale Yellow:4 Magenta Rose. F_3 and F_4 generations of this cross have been grown to check upon the occurrence of flaking. Flower-color mutations were observed, but not in excess of what would be expected of any *M. jalapa* material.

(7) Yellow×Rose Pink. This cross gave results similar to the original of KIERNAN and WHITE (1926), except that in my work, Yellow of the *YYrprp* type was used instead of the *YYrr* of the former work. These Yellows are indistinguishable, except in breeding experiments. The F_1 Rose Red (see table 3–12) gave an F_2 of Crimson, Yellow, Rose Pink, Flesh Pink, Scarlet Red, Rhodamine Purple, Pale Yellow, Light Pink and Rose Red; in an approximate 1:1:1:1:2:2:2:2:4 ratio (see table 1–7).

(8) Yellow×Flesh Pink. The F_1 heterozygote of this cross is Pale Yellow (see table 3–17 or 18), indistinguishable from that of the Yellow× Dominant White cross, though theoretically it shows more pink. In the F_2, Yellow, Pale Yellow, and Flesh Pink were obtained closely approximating a 1:2:1 ratio. The parents in my crosses had the genotypes *YY rp rp* and *yy rp rp*, respectively. As a proof of this, no Whites occurred in the F_2. White would be expected when either parent carries the factor *r* instead of *rp*.

(9) Yellow×Recessive White. In the F_2 an approximate 1:2:1 ratio of Yellows, Pale Yellows, and Whites was obtained (see table 1–9). The Yellow parent was derived from a strain homozygous for *r* (see table 2–3). This cross was used to check on the genotypes of the Yellows.

(10) Dominant White×Rose Pink. The results are similar to those of

KIERNAN and WHITE (1926). The Light Pink heterozygote (see table 3–19) is difficult, or impossible, to distinguish from those of the Dominant White×Flesh Pink, and Rose Pink×Recessive White crosses (see table 1–11, 14). The results of this cross are given in table 1–10.

(11) Dominant White×Flesh Pink. This cross gave an F_1 Light Pink (see table 3–21), probably less intense than that from the Dominant White ×Rose Pink cross. In the F_2, Whites, Light Pinks, and Flesh Pinks are obtained in an approximate 1:2:1 ratio (see table 1–11).

(12) Dominant White×Recessive White. As expected, this cross gave only White in the F_1 and F_2 (see table 1–12). For the genotypes of White see tables 2–5, 6 and 3–3.

(13) Rose Pink×Flesh Pink. The F_1 heterozygote, Rose Pink, is only slightly, if any, lighter than the Rose Pink parent (see table 3–22 and 2–7, respectively). In the F_2 Rose Pink and Flesh Pink were obtained closely approximating a 3:1 ratio (see table 1–13), showing that factor *Rp* for Rose Pink is dominant over *rp*, the factor for Flesh Pink.

(14) Rose Pink×Recessive White. The F_1 heterozygote of this cross is difficult, or impossible, to distinguish from the Light Pink of other crosses, as for instance the cross, Dominant White×Rose Pink. Upon selfing, however, it gives a close 1:2:1 ratio of Rose Pink, Light Pink, and White (see table 1–14).

(15) Flesh Pink×Recessive White. Flesh Pink is obtained in the F_1, indicating that the factor *rp* for Flesh Pink is dominant over *r*, the factor for Recessive White. Only a few F_2 plants have been grown from this cross, but of these a somewhat poor approximation to a 3:1 ratio of Flesh Pink and White was obtained (see table 1–15). Some flaking occurred in the F_1 and F_2 progeny of this cross.

(16) Dominant White×Light Rosaline Purple. This is the only cross involving Light Rosaline Purple which has yielded an F_2 to date. In the F_1 a purple lighter than the purple parent is obtained. It is analogous to the heterozygous stock from which Light Rosaline Purple was first obtained, namely: Pale Rosaline Purple. Upon selfing, the F_1 heterozygote gives an F_2 of Light Rosaline Purple, Pale Rosaline Purple, and White, in approximately a 1:2:1 ratio (see table 1–16).

The behavior of Light Rosaline Purple indicates that it carries a factor allelic to the *R* series. It breeds true, and on the basis of the observations already made on this new flower type, the factor symbol *Rpl* is given it. The F_1 of a cross, Crimson×Light Rosaline Purple, is of a darker shade of Rhodamine Purple than that of the cross, Crimson×Rose Pink. In the cross, Rose Pink×Light Rosaline Purple, Pale Rosaline Purple, apparently indistinguishable from that of the Dominant White×Light Rosaline Purple, is obtained. The factor *Rpl* gives more color, both in the presence

and in the absence of *Y*, than does *Rp*, or any other factors of the *R* series. It is practically dominant over the factors *Rp* and *R*, and probably should be listed at the top of the *R* series.

FLOWER-COLOR MUTATION

It is not uncommon to see a plant of *M. jalapa* bearing two distinctly different flower colors, in the same, or in different flowers. In cases where mutation occurs in self-colored plants, there is generally a preponderance of flowers of the same color, with a few of a different, or mutant color. These few are borne on one (rarely more than one) terminal branch, indicating that somatic mutation took place late in the growth of the plant, giving a new flower color. In some cases not all the flowers of such a branch are self-colored. A single blossom may show part mutant and part non-mutant color, indicating that the whole of the growing tip of the branch was not involved in the mutation. This suggests the problem of "flaking" to which MARRYAT (1909) and CORRENS (1910) gave so much of their attention while working with this species. The phenomenon will not be discussed further in this paper.

Of the 5,000 plants (slightly more) of *M. jalapa* grown since this investigation began, 21 self flower-color mutations have been observed and recorded. Most of the observations have been confined to material with known breeding behavior.

The somatic mutations observed are as follows:

(1) White with Light Pink branch (*R* to *Rp*) 6 cases
(2) White with Light Pink branch (*R* to *rp*) 2 cases
(3) White with Pale Rosaline Purple branch (*R* to *Rpl*) 3 cases
(4) White with Light Buff branch (? to ?) 3 cases
(5) Rose Pink with Light Pink branch (*Rp* to *R*) 1 case
(6) Crimson with Orange Red branch (*R* to *r*) 4 cases
(7) Flesh Pink with Light Pink branch (*rp* to *R*) 2 cases

It is very probable that Flesh Pink mutates to Rose Pink (*rp* to *Rp*), and that Dominant White and Flesh Pink both mutate to Recessive White (*R* and *rp* to *r*), but that in the latter two cases the mutation is covered by dominancy. It is probable that the total number of somatic mutations in self-flower color is appreciably higher than the observed number (21 per 5,000 individuals), probably one mutation per sixty individuals. It should be stated that the observed mutation frequency is noticeably higher in some flower-color classes than in others; and it may be higher in one line than in another of the same genotype. With one exception I have found the mutant characters in the heterozygous condition. In that case I obtained four seeds from a branch bearing presumably Rose Pink flowers on a plant whose other flowers were White. The three plants obtained

from these seed were classified as Rose Pink. If this be true, it would mean that double mutation (involving genes in homologous chromosomes) had taken place. An F_3 of these plants may reveal even yet, that this was a single mutation, and that a Light Pink mutation was mistaken for Rose Pink.

In addition to somatic mutation, a few cases of what appears to be gametic mutation have been found. Pale Rosaline Purple (1 plant) was obtained in the F_2 population of a cross, Crimson (*YY Rp Rp* type) × Dominant White, out of a total of 90 plants (in 1931). Another case of the same kind occurred in 1933 in an F_2 population of a cross, Dominant White × Flesh Pink, giving Violet-Purple, the only instance of this color class. Just what this type will give upon selfing is not known, but like Pale Rosaline Purple, it will probably give rise to a new true-breeding color variety, analogous to Light Rosaline Purple.

DISCUSSION

Prior to the discovery of the factor *rp*, it was comparatively easy to identify the individuals in a hybrid population of *M. jalapa* plants as to their genotypes, except for the Whites. Since its discovery it is difficult, and in most cases impossible, to distinguish the flower-color classes, except by breeding tests. Yellow, formerly thought to be of only one genotype, is known to have three. So far as I am concerned they are not distinguishable, though theoretically they should be. Those having the factor *rp* in their complex should show a trace of pink, not expected in the original Yellow with *r* instead of *rp*. Similarly in Crimson there are three known genotypes (and probably more will be had), depending on the factors of the *R* series present, whether *R* itself, or its allele *Rp*, in the homozygous condition; or both in the heterozygous condition. Each has a distinct action in modifying *Y* (factor for color base) to red, but I find that the phenotypic expression from one type to another, is not sufficiently affected to distinguish between them accurately. With the discovery of the factor for Light Rosaline Purple, tentatively designated *Rpl*, which also possesses the power to modify *Y* to red, a third homozygous Crimson type is postulated, which theoretically will show more purple color than those already known, but actually it may appear indistinguishable. Its behavior in the cross, Crimson (of the *YYRpRp* type) × Light Rosaline Purple, giving the heterozygote, Rhodamine Purple, darker than that of the Crimson × Rose Pink cross, indicates that the factor *Rpl* may show up distinctly in Crimson where reddening is at its maximum. This would bring the total of Crimson genotypes up to six, and would increase the number of heterozygotes with Crimson proportionally. As in Crimson and Yellow, there is more than one type recognized of Rose Pink, Flesh Pink, and White.

Factors *R*, *Rp*, and *Rpl* all produce reddening in the presence of *Y*. In addition to reddening, *Rp* produces pinking and slight purpling. In addition to reddening, pinking and slight purpling, *Rpl* produces deeper purpling, thus combining and exceeding the action of the first two, *R* and *Rp*. This justifies naming it at the top of the *R* series, with *Rp* and *R* next in order. *rp* like *r* does not produce reddening in the presence of *Y*, but it is dominant to *r*, as revealed in the cross, Flesh Pink×Recessive White. In turn *rp* is recessive to *Rp*, or nearly so as seen from the cross, Rose Pink×Flesh Pink. On the basis of their behavior, the alleles of the *R* series may be arranged as follows, beginning at the top: *Rpl*, *Rp*, *R*, *rp*, and *r*. Upon further investigation of the new self-color types, Violet-Purple and Light Buff, the series may be lengthened.

No evidence has been accumulated to show that any of the alleles of the *R* series are stable. No observations have been made of *r* changing to *rp*, or *rp* to *Rp*, but since *rp* is dominant to *r*, and *Rp* to *rp*, these reverses could not have been seen at once, and would be lost in the population. Of the mutations in self flower-color so far observed, 14 represent changes from *R* to some allele, except perhaps in the case where Violet-Purple occurred. *Rp* stands next in the number of changes observed with 5; and *rp* third with 2. The individuals in my populations having *Rp* and *rp* in their complex have been as numerous as those with *R*, but have not shown as great a number of changes. *Rpl*, factor for Light Rosaline Purple, has arisen from *R* three times, but not once from *Rp* to date. Light Buff has arisen from White plants.

KIERNAN and WHITE's postulation (1926) that the factor *Rp* arose by mutation from *R* has been verified in this investigation. At least 6 cases have been observed where White Plants have born Light Pink flowers. Seeds obtained from these branches yield Rose Pink, Light Pink, and White plants. *Rp* and *R* have both reverted in my material to *rp* for Flesh Pink. Since practically all of my material was grown from stocks obtained from KIERNAN and WHITE, they must have had the same phenomenon too. Of the seed which I obtained from them, one package gave Flesh Pink plants. It was, however, labeled "Light Pink," indicating that they had Flesh Pink in their stocks, but that they did not distinguish it from Light Pink.

SUMMARY

1. Crosses and reciprocal crosses have been made of all the known true-breeding color varieties of *M. jalapa*, namely: Crimson, Yellow, Dominant White, Rose Pink, Flesh Pink, Recessive White, and Light Rosaline Purple. The latter has not been investigated thoroughly.

2. Data are presented on the spontaneous occurrence of varieties, Rose Pink, Flesh Pink, Light Rosaline Purple, Violet-Purple, and Light Buff.

The breeding behavior of Flesh Pink is given in detail, while Light Rosaline Purple, Violet-Purple, and Light Buff have yet to be investigated more thoroughly.

3. The whole range of flower-color classes is interpreted as being due to the action and interaction of factors at two loci, a *Y* (color base) and an *R* (modifying) locus.

4. The factors for Flesh Pink and Light Rosaline Purple have been isolated and designated *rp* and *Rpl*, respectively. These factors have been found to be alleles of the *R* series.

5. The alleles of the *R* series have been arranged as follows, beginning at the top: *Rpl*, *Rp*, *R*, *rp*, and *r*.

6. In the presence of *Y*, *Rpl* covers the action of *R* by producing reddening, and that of *Rp* by producing reddening, pinking, and slight purpling, and exceeds their action by giving more intense purpling.

8. *rp*, factor for Flesh Pink, is recessive to *Rp*, produces Light Pink in the presence of *R*, and is dominant to *r*. It does not possess the power to modify *Y* to red.

9. Factor *R* is probably less stable than any of its alleles.

10. Somatic mutation is common, taking place in the late ontogeny of the plant. Cases of gametic mutation have been observed, it is thought, but they are more rare.

11. The discovery of factor *rp* gives further complications to recognizing the various color classes in a population.

12. On the basis of related genotypes, 9 heterozygous groups have been recognized.

ACKNOWLEDGMENT

The writer is indebted to Dr. ORLAND E. WHITE for numerous suggestions and criticisms given during this study.

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THE INHERITANCE OF THE CRESCENT AND TWIN SPOT MARKING IN XIPHOPHORUS HELLERI

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Received January 12, 1934

INTRODUCTION

The viviparous teleost, *Xiphophorus helleri*, was first described and named by the naturalist, HECKEL, in 1848. The genus Xiphophorus has the lower rays of the caudal fin in the males prolonged into a long sword-shaped appendage which is sometimes as long as the rest of the fish. The anal fin of the male is modified into an intromittent organ, the gonopodium.

The classification of *Xiphophorus helleri*, according to HUBBS (1924) is as follows:

Order: Cyprinodontes
Family: Poeciliidae
Sub-family: Poeciliinae
Tribe: Xiphophorini
Genus: Xiphophorus

According to REGAN (1913), these are three species:

1. *X. montezumae*
2. *X. helleri*
3. *X. brevis*

This paper deals with *Xiphophorus helleri*, variety *rachovii*. This variety was thought by REGAN in 1911 to be a new species which he called *X. rachovii* in honor of ARTHUR RACHOW of Hamburg, Germany, who sent him the specimens. These fish had a pair of conspicuous blackish spots, one above and one below, at the base of the caudal fin. In his revision in 1913, however, REGAN considered them as a variety of *X. helleri*. I have found that some of these fish show two spots at the base of the caudal fin, while other animals have the spots continuous at the base of the caudal fin to give the appearance of a crescent. I have indicated the one with spots as twin spot, and the other as crescent.

BREEDING TECHNIQUE

In breeding *Xiphophorus helleri*, the fish were kept in small balanced aquaria. Unmated females were used for all crosses. This stock of immature virgin females was kept intact by removing the young fish which showed a thickening of the fore-margin of the anal fin. One or more females were placed with a single male. When the female showed signs of pregnancy,

she was removed to a tank well stocked with floating plants and fed with live food. After the birth of the fish, the female was removed and the young left in the aquarium.

Each young fish was placed in a 50 cc beaker and carefully examined with a hand lens. In transferring all fish, even the mature, I have followed the practice of keeping them in water. Either a specially constructed glass

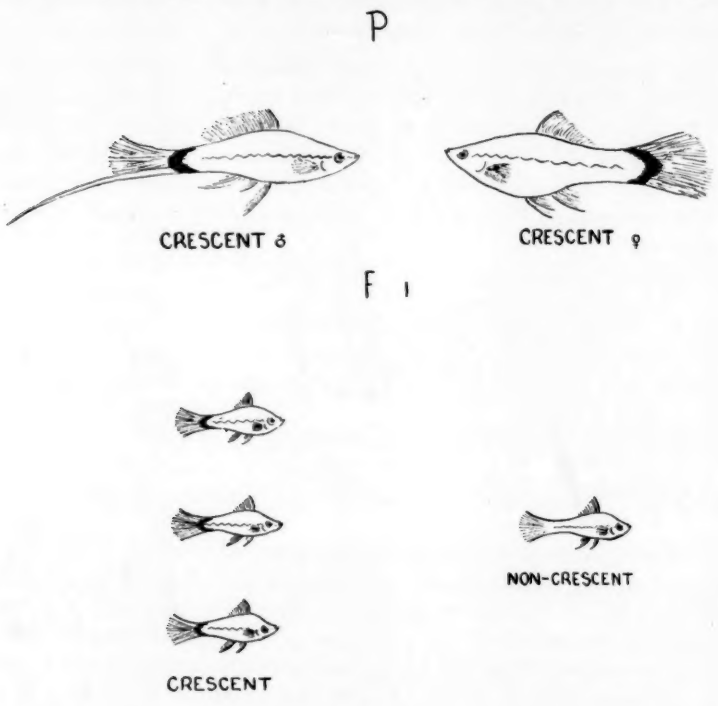

FIGURE 1.—The mating of a heterozygous crescent male with a heterozygous crescent female.

cup-shaped container having a handle about twelve inches long or a glass beaker served as a transfer medium. After the examination of each fish, the young were returned to the container in which they were born. These young were allowed to remain in this container for at least two months before being removed to a larger one.

EXPERIMENTAL DATA

The stock used in the following experiments was obtained from F. W. SCHUMACHER, 753 Center Street, Jamaica Plain, Massachusetts, with the

exception of a crescent male (Series 13-1) which was purchased from WILLIAM TRICKER, INC., Saddle River, New Jersey. The work was begun in October, 1930.

A male crescent was mated with three females, two of which showed no crescent marking. The following experiments will prove the genotype of the male to be *Cc Pp* and the female crescent *Cc PP*. The results of the mating of the crescent male with the crescent female is shown in the following table (see also figure 1).

TABLE 1

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT		NON-CRESCENT	
				MALES	FEMALES	MALES	FEMALES
11–1	F_1	February 9, 1931	1	3	5	2	
11–1	F_1	July 22, 1931	2	1	2		2
Total					11		4
Expected on basis of *Cc*×*Cc*—3:1					11:25		3:75

The factor for crescent marking is evidently dominant and the parents are heterozygous for this factor.

The genotypes of brood 1 of the F_1 as regards the crescent marking was next determined. A non-crescent male of F_1 mated with a crescent female of F_1 gave the results shown in table 2 (see also figure 2. In this drawing, the reciprocal cross is indicated).

TABLE 2

SERIES NUMBER	GENERATION	BROOD NUMBER	DATE	CRESCENT	NON-CRESCENT
21–1	F_2	1	March 24, 1932	5	2
21–1	F_2	2	April 24, 1932	7	6
21–1	F_2	3	May 22, 1932	10	9
21–1	F_2	4	July 9, 1932	16	24
21–1	F_2	5	August 23, 1932	9	9
Total				47	50
Expected on basis of *Cc*×*cc*—1:1				48.5	48.5

This crescent female is, therefore, heterozygous for the crescent factor. The same male was mated with another crescent female giving the results in table 3.

TABLE 3

SERIES NUMBER	GENERATION	BROOD NUMBER	DATE	CRESCENT	NON-CRESCENT
21–2	F_2	1	May 22, 1932	19	0
21–2	F_2	2	June 24, 1932	13	0
21–2	F_2	3	August 7, 1932	12	0
Total				44	0
Expected on basis of *CC*×*cc*				44	0

The female is, therefore, homozygous for the crescent factor, while the former female is heterozygous.

The other non-crescent male of brood 1 of F_1 was mated with another crescent female, giving the results shown in table 4.

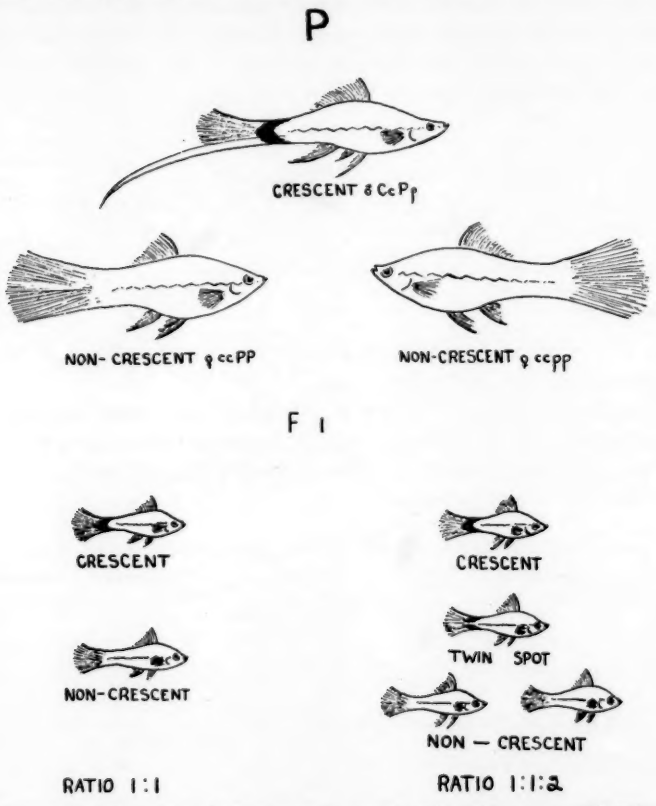

FIGURE 2.—The mating of a heterozygous crescent male with non-crescent females.

TABLE 4

SERIES NUMBER	GENERATION	BROOD NUMBER	DATE	CRESCENT	NON-CRESCENT
22–1	F_2	1	April 24, 1932	3	4
22–1	F_2	2	May 19, 1932	3	5
22–1	F_2	3	June 28, 1932	16	15
22–1	F_2	4	August 15, 1932	10	7
Total				32	31
Expected on basis of *Cc*×*cc*—1:1				31.5	31.5

This female is heterozygous for the crescent factor. The genotypes of the three females may be represented by *Cc*, *Cc*, and *CC*. *C* = dominant crescent gene and *c* = the recessive non-crescent gene.

The two female non-crescents of the brood died when half grown, so that the male crescents were mated with non-crescent females obtained in the breeding of *Xiphophorus helleri*, which showed no crescent markings. The males were removed from the brood at the appearance of the thickening of the anal fin. The males were kept in a twenty gallon tank and the females in a twenty-five gallon tank.

The data on the breeding of the two non-crescent animals is as follows:

TABLE 5

SERIES NUMBER	GENERATION	FEMALE NUMBER	DATE	BROOD NUMBER	NUMBER OF NON-CRESCENT YOUNG
12	F_1	1	April 30, 1931	1	10
12	F_1	1	July 22, 1931	2	5
12	F_1	2	April 14, 1931	1	23
12	F_1	2	July 13, 1931	2	10
12	F_1	3	August 17, 1931	1	15
Total					63

Of the 63 non-crescent offspring, only 51 lived to reach maturity, even though they were kept in fairly large containers. Of the 51, 20 were females and 31 were males.

The male crescents of the F_1 of series 11-1 were mated with females of series 12. There were only two crescent males since one died. One crescent male mated with a non-crescent female gave the results in table 6.

TABLE 6

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	NON-CRESCENT
23–1	F_2	March 24, 1932	1	22	0
23–1	F_2	April 30, 1932	2	26	0
23–1	F_2	June 20, 1932	3	18	0
Total				66	0
Expected on basis of $CC \times cc$				66	0

This male is evidently homozygous for the crescent factor.

The other male crescent mated with a female of series 12 gave the results in table 7.

TABLE 7

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
24–1	F_2	August 17, 1932	1	5	1	8
24–1	F_2	October 6, 1932	2	17	6	24
24–1	F_2	December 6, 1932	3	8	3	16
Total				30	10	48
Expected on basis of 3:1:4				33	11	44

This same male crescent mated with another female of series 12 gave the results in table 8 (see also figure 2).

TABLE 8

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
24–2	F_2	June 20, 1932	1	1	2	1
24–2	F_2	August 5, 1932	2	2	1	1
24–2	F_2	September 7, 1932	3	2	2	6
24–2	F_2	November 15, 1932	4	0	0	2
Total				5	5	10
Expected on basis of 1:1:2				5	5	10

This same male crescent mated with a third female of series 12 gave the results in table 9 (see also figure 2).

TABLE 9

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
24–3	F_2	August 23, 1932	1	1	0	1
24–3	F_2	October 6, 1932	2	1	1	4
Total				2	1	5
Expected on basis of 1:1:2				2	2	4

This male is heterozygous for the crescent factor. Of the five crescent fish tested in the F_1 of series 11–1, three are heterozygous and two are homozygous for crescent marking. The genotype of the females may be represented by *Cc*, *Cc*, and *CC*,—the males by *CC* and *Cc*.

There is, however, another factor that is introduced—that of twin spots. In this case, the crescent at the base of the tail fin is incomplete. The black pigment appears only in the dorsal region, and the ventral region, and the area between the two is without pigment, thus giving the effect of two spots. Twin spots appeared in the last case (see tables 7, 8, and 9) when the crescent male was bred with females from series 12.

Another crescent male (purchased from TRICKER'S) when mated with a female from series 12, gave the results in table 10, in which no twin spot fish appeared (see also figure 2).

TABLE 10

SERIES NUMBER	GENERATION	DATE	NUMBER	CRESCENT	NON-CRESCENT
13–1	F_1	May 19, 1932		8	7
13–1	F_1	August 13, 1932	2	9	11
Total				17	18
Expected on basis of *Cc*×*cc*—1:1				17.5	17.5

The results in table 6 in which a crescent male again was mated with a female from series 12 showed no twin spots in the offspring. It would, therefore, appear that there were two kinds of non-crescent animals as regards this factor.

This twin spot factor was earlier observed when the original male crescent (*P*) of parent series 11–1 was mated with non-crescent females. The following results were obtained:

TABLE 11

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	NON-CRESCENT
11–2	F_1	April 10, 1931	1	0	3
11–2	F_1	May 10, 1931	2	5	2
11–2	F_1	June 22, 1931	3	6	6
11–2	F_1	July 31, 1931	4	0	5
Total				11	16
Expected on basis of *Cc*×*cc*—1:1				13.5	13.5

The twin spots were not distinguished when the young were born. Evidently the figures listed for non-crescent include some animals having twin spots, because on December 14, 1931, there were only 6 survivors of these 27 animals. Observation showed that one had a complete crescent marking, two had the crescent marking interrupted to give the twin spot effect, and three had no crescent or spotted marking on the tail fin. The two twin spot animals and the one non-crescent died before the sex was determined. The fish remaining were:

Crescent	Non-crescent
1 ♀	1 ♀, 1 ♂

When this same male (*P*) was mated with another non-crescent female, the following results were obtained.

TABLE 12

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	NON-CRESCENT
11–3	F_1	May 8, 1931	1	5	11
11–3	F_1	July 4, 1931	2	3	2
Total				8	13
Expected on basis of *Cc*×*cc*—1:1				10.5	10.5

December 14, 1931, there were 12 survivors, as follows:

Brood Number	*Crescent*	*Twin Spot*	*Non-Crescent*
1	2 ♀	3 ♀	3 ♀, 1 ♂
2	1 ♀	2 ♀	0
Total	3 ♀	5 ♀	3 ♀, 1 ♂

A twin spot male mated with a non-spot female gave the following results:

TABLE 13

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
15–1	F_1	July 2, 1931	1	1♀	1♀	8♀, 1♂
15–1	F_1	August 10, 1931	2	1♀	0	1♂
15–1	F_1	May 22, 1932	3	9	7	20
15–1	F_1	July 1, 1932	4	10	8	20
15–1	F_1	August 15, 1932	5	9	7	15
15–1	F_1	October 10, 1932	6	4	5	9
Total				34	28	74
Expected on ratio 1:1:2				34	34	68

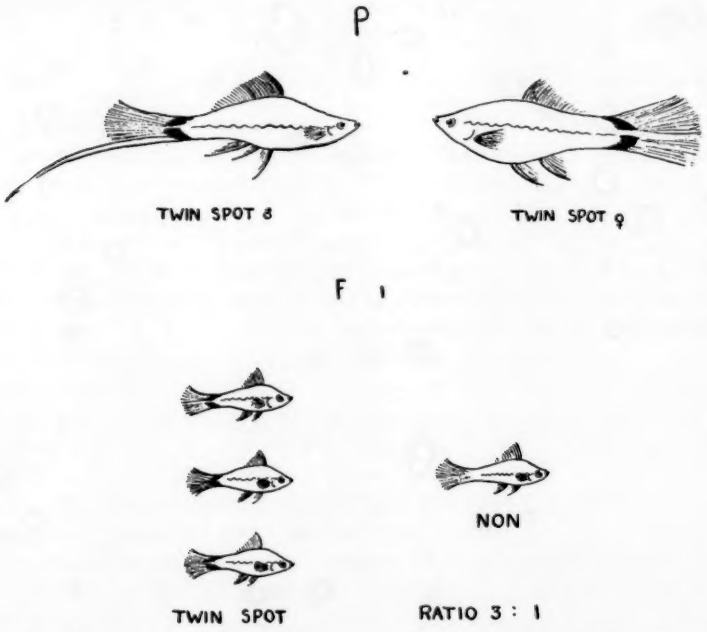

FIGURE 3.—The mating of a heterozygous twin-spot male with a heterozygous twin-spot female.

The genotypes of brood 1 were next demonstrated. The twin spot female mated with a twin spot male gave the results in table 14 (see also figure 3).

This same twin spot male mated with a non-crescent female of brood 1 gave the results in table 15 (see also figure 4).

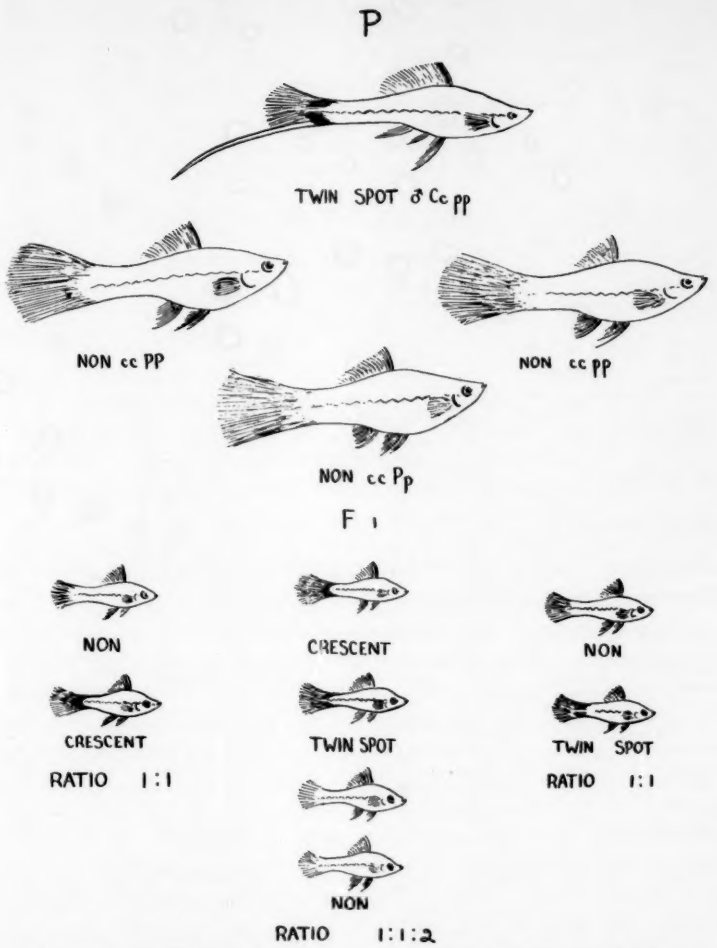

FIGURE 4.—The mating of a heterozygous twin spot male with non-spot females.

TABLE 14

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	TWIN SPOT	NON-CRESCENT
25–1	F_2	July 6, 1932	1	4	3
25–1	F_2	August 15, 1932	2	10	3
25–1	F_2	September 26, 1932	3	18	7
25–1	F_2	November 14, 1932	4	8	3
Total				40	16
Expected on basis of 3:1				42	14

TABLE 15

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
25–2	F_2	August 5, 1932	1	4	3	9
25–2	F_2	September 18, 1932	2	8	7	20
25–2	F_2	November 13, 1932	3	5	6	9
Total				17	16	38
Expected in ratio 1:1:2				17.5	17.5	35

This same twin spot male mated with another non-crescent female of brood 1 gave the results in table 16 (see also figure 4).

TABLE 16

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	TWIN SPOT	NON-CRESCENT
25–3	F_2	August 18, 1932	1	2	3
25–3	F_2	September 27, 1932	2	8	10
25–3	F_2	November 23, 1932	3	12	14
Total				22	27
Expected on basis of 1:1				24.5	24.5

The crescent female of brood 1, series 15-1, mated with a male non-crescent of the same brood gave the following results:

TABLE 17

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
26–1	F_2	June 14, 1932	1	2	3	5
26–1	F_2	August 3, 1932	2	4	3	7
26–1	F_2	September 15, 1932	3	7	6	20
Total				13	12	32
Expected on ratio 1:1:2				14.5	14.5	29

When born, the ration of brood 1 was 8 non-crescent and 2 crescent. The twin spot characteristic was clearly visible a month later. Likewise brood 2, when born, consisted of 4 crescent, 10 non-crescent, and brood 3 consisted of 7 crescent, 26 non-crescent.

A non-crescent female of brood 1, series 15–1, mated with the original male (*P*) of series 11–1, proven heterozygous for crescent marking, gave the results in table 18.

TABLE 18

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
11–4	F_2	June 13, 1932	1	1	1	3
Expected on ratio 1:1:2				1.25	1.25	2.50

A female twin spot mated with a male non-spot gave the results in table 19 (see also figure 4. In this drawing, the reciprocal cross is indicated).

TABLE 19

SERIES NUMBER	GENERATION	DATE	BROOD NUMBER	CRESCENT	TWIN SPOT	NON-CRESCENT
16–1	F_1	March 21, 1932	1	28	0	26
16–1	F_1	May 4, 1932	2	11	0	15
16–1	F_1	June 13, 1932	3	11	0	13
Total				50		54
Expected on ratio 1:1				52		52

These fishes had a decided crescent marking, and there were no twin spot animals.

DISCUSSION

Crescent and twin spot marking

An examination of the preceding results shows clearly that the factor for crescent marking is dominant over that for non-crescent or lack of marking. There must be, however, two different types of non-crescent animals, since a heterozygous crescent mated with a non-crescent will give crescent and non-crescent offspring in a ratio of 1:1, as results in tables 2 and 4 indicate. On the other hand, a heterozygous crescent mated with a non-crescent gives crescent, twin spot, and non-crescent animals (see tables 7, 8, and 9). A factor is introduced which evidently interacts with one present to give a different phenotype. If the factors for crescent, twin spot, and non-crescent were triple alleles, we might expect the crescent factor to be dominant over twin spot and non-crescent, and, in turn, twin spot dominant over non-crescent, as is the case in the fresh water fish, *Aplocheilus latipes* (AIDA 1921), in which the three colors, brown, variegated red, and red form a triple allelic series. Any two, when crossed, show in the F_2 a 3 to 1 segregation. Brown is dominant to variegated red as well as to red, while variegated red is dominant to red. A similar mode of inheritance was described by IBSEN (1919) in respect to black, tortoise, and red color in guinea pigs which are produced respectively by complete extension, partial extension, and non-extension of black pigment.

The three factors, crescent, twin spot, and non-crescent do not act in this way in Xiphophorus and, consequently, are not allelic. It is clearly a case of interaction of factors in which the factors belong to different allelic pairs. The crescent characteristic is due to two factors, one for color *C* and pattern *P*. The twin spot characteristic is due to the same pigment *C* but the pattern is reduced (*p*). Therefore, the genotype of the homozygous crescent could be represented by *CCPP*, the genotype of the homozygous twin spot could be represented by *CCpp*, and consequently, the homozygous non-crescent or non-spot animal could be *ccPP* or *ccpp*.

An examination of the results given in the tables of the various crosses will determine whether or not this is the correct explanation.

In table 1, the parents were proven to be heterozygous for the crescent factor. Twin spots did not appear in the F_1. However, in F_2, twin spots do appear (see tables 7, 8, and 9) so that one of the parents must be heterozygous for *P* as well as for *C*. The parent heterozygous for *P* is the male, since tables 11 and 12 give the results of the mating of this male with non-crescent females in which twin spot offspring appear. This male then would have to be heterozygous for factor *P*. The genotype of the male parent in table 1 is, therefore, *CcPp*, and the genotype of the female parent is *CcPP*.

Diagram to illustrate series 11–1, table 1.

P *CcPP* ♀ × *CcPp* ♂

↓

Gametes	♂ *Cp*	*Cp*	*cP*	*cp*
♀ *CP*	*CCPP* Crescent	*CCPp* Crescent	*CcPP* Crescent	*CcPp* Crescent
F_1 *cP*	*CcPP* Crescent	*CcPp* Crescent	*ccPP* Non-Crescent	*ccPp* Non-Crescent

Phenotypic Ratic in F_1		Genotypic Ratio in F_1
3 *C– P–*	crescent	1 *CCPP* crescent 1 *CCPp* crescent 2 *CcPP* crescent 2 *CcPp* crescent
1 *ccP–*	non-crescent	1 *ccPP* non-crescent 1 *ccPp* non-crescent

The phenotypic ratio is in agreement with results in table 1.

Results in table 2 indicate that the genotype of the two fish must be either

CcPP crescent × *ccPP* non-crescent
or
CcPp crescent × *ccPP* non-crescent
or
CcPP crescent × *ccPp* non-crescent

to give a 1:1 ratio of crescent and non-crescent offspring in F_2. The crescent fish cannot have the genotype *CCPP* or *CCPp*, since the F_2 would be all crescent. Neither could the mating be *CcPp* crescent × *ccPp* non-crescent—since twin spot fish would be expected in the F_2 in the ratio of 3 crescent, 1 twin spot and 4 non-crescent. Of the 97 fish, 8 should be twin spot and no twin spot fish were obtained.

Table 3 indicates that the genotypes of the two parents must be either

CCPP crescent × *ccPP* non-crescent
or
CCPp crescent × *ccPp* non-crescent
or
CCPp crescent × *ccPP* non-crescent

to give all crescent offspring. The genotypes of the two parents could not be *CCPp*×*ccPp* since the offspring would be 3 (*C*−*P*−) crescent to 1 (*C*−*pp*) twin spot. The crescent in table 2 is heterozygous for factor *C*, while that in table 3 is homozygous for this factor.

Results in table 4 show that this mating is similar to that in table 2, and there are the same genotypic possibilities. Results in table 6 are similar to those in table 3, and the genotypic possibilities are the same.

Results in tables 7, 8, and 9 indicate that the crescent parent must have the genotypic formula *CcPp* in order to have twin spot offspring. The non-crescent females have either the genotypes *ccPp* or *ccpp*. They could not have the genotype *ccPP*, since in this case no twin spot offspring could appear. If the genotype of the non-crescent female is *ccPp*, the expected ratio would be 3 crescent, 1 twin spot, and 4 non-crescent, while if *ccpp*, the ratio would be 1 crescent, 1 twin spot, and 2 non-crescent. The figures in table 7 seem to indicate that this female is *ccPp*, since the results approximate a 3:1:4 ratio. The female in table 8 must have the genotype *ccpp* since 5 twin spot offspring were obtained out of a total of 20. The females in tables 7 and 8 are, therefore, genotypically different as regards the *P* factor as the ratio of the results shows. The female in table 9 also has the genotype *ccpp*, since the results, although small, indicate a 1:1:2 ratio.

In tables 11 and 12, the male parent (*P*) series 11–1 is proven to have the genotype *CcPp*. The non-crescent females have either *ccPp* or *ccpp* genotypes. Figures would tend to indicate, particularly in table 12, that the genotype of the non-crescent female is *ccpp*, since there are 5 twin spot fish to 3 crescent. If the parent had the *ccPp* genotype, the ratio should be 3 crescent to 1 twin spot. The 5 twin spot to 3 crescent ratio more closely approximates the 1:1 ratio of twin spot and crescent expected if the genotype of the female is *ccpp*.

It is possible to account for the fact that, from the mating of a non-crescent individual with a twin spot, crescent offspring are obtained. In table 13, the twin spot male parent must be *Ccpp*, since non-crescent offspring are obtained. The only genotype for the non-crescent female parent to have is *ccPp* to give crescent and twin spot offspring, as the diagram below indicates.

P	*Ccpp* ×	*ccPP*	*ccPp*		*ccpp*
	♂	♀	♀		♀
	Twin spot	Non	Non		Non
		↓	↓		↓
	gametes ♀	*cP*	*cP*	*cp*	*cp*
	♂	*CcPp*	*CcPp*	*Ccpp*	*Ccpp*
	Cp	Crescent	Crescent	Twin spot	Twin spot
F_1					
	cp	*ccPp*	*ccPp*	*ccpp*	*ccpp*
		Non-crescent	Non-crescent	Non-crescent	Non-crescent

Since the twin spot female of the F_1 is *Ccpp*, the genotype of the male twin spot must be *Ccpp* to give the results in table 14 of twin spot to non-crescent offspring in the ratio of 3:1. The non-crescent female in table 15 must have the genotype *ccPp*, since the ratio obtained is 1 crescent, 1 twin spot to 2 non-crescent when mated with this one male those genotype is *Ccpp*. Likewise, the genotype of the female in table 16 must be *ccpp* to give a ratio of 1 twin spot to 1 non-crescent offspring when mated with this same male.

The crescent female of the F_1 of series 15–1 has the genotype *CcPp*, and mated with a non-crescent of the F_1 gives offspring which approximate a 1:1:2 ratio rather than a 3:1:4 ratio of crescent, twin spot, and non-crescent fish, as is indicated in table 17. The non-crescent genotype is, therefore, *ccpp* rather than *ccPp*. The non-crescent female of F_1 which was mated with the male (*P*) of series 11–1 whose genotype was proven to be *CcPp* probably has also the genotype *ccpp*. Although too small for certainty, the figures in table 18 seem to be more in accord with the 1:1:2 ratio, which would indicate the non-crescent genotype to be *ccpp*.

The results in table 19 of the cross of a twin spot and non-crescent to give only crescent and non-crescent offspring may be explained as follows. The twin spot must have the genotype *Ccpp*. The only other possible genotype is *CCpp*, and in this case there could be no non-crescent offspring. The only genotype the non-crescent parent can possibly have is *ccPP* to give the results in table 19 of crescent and non-crescent offspring.

It has been found possible to explain the results of all crosses on the basis of interaction of factors. The crescent marking is due to two factors, *C* which evidently indicates color pigment, and *P* the pattern. The crescent phenotype is then $C-P-$, in which *P* represents a supplementary gene which acts only in conjunction with *C* to produce this crescent marking. When *P* is found in a homozygous recessive condition *pp* in the presence of *C*, the twin spot phenotype appears $C-pp$, as if the patterns were not as extended or complete as in the previous condition $C-P-$. In the non-

crescent animals, color never appears and the factor for pattern is concealed. The genotypes of these fish are *ccpp*, *ccPP*, and *ccPp*.

The hair pattern determiner demonstrated by CASTLE in guinea pigs is similar to this in which a supplementary gene acts only in conjunction with some other gene to bring about a modification. The wild agouti color of the hair of certain guinea pigs is due to the fact that pigment is distributed along the length of each hair in a definite pattern. The tip of a single hair is black followed by a band of yellow, while most of the proximal part is a leaden color. The distribution of the pigment is such as to give the characteristic gray or "agouti" color to the coat when taken as a whole. CASTLE showed that upon crossing a solid black guinea pig, possessing pigment but no pattern (*CCaa*) with a white albino pig having no pigment (*ccAA*), the offspring reverted to the ancestral agouti or pattern type (*CcAa*) thus proving that the pattern must be carried in this case by the white or albino guinea pig as a factor independent of the color necessary for its expression. The same is true of Xiphophorus, the non-crescent animal carrying the factor which is necessary for interaction with the color factor to produce either crescent or twin spot marking.

The spotting of piebald mice is another illustration of this same type of interaction of factors. CUÉNOT discovered that such spotting is due to the absence of a uniformity gene which, if present, causes color to be uniformly distributed over the entire coat. Both of these independent allelic genes are transmitted through albino animals, although in the albino both are hidden due to the absence of pigment upon the presence of which their visibility depends, just as in Xiphophorus the non-crescent may carry *pp* or *P*— which produces different phototypes in the presence of *C*.

There are numerous other illustrations of the interaction of factors to produce different phenotypes. There are in both rabbits and mice factors which interact to produce various coat colors, spotting, clumping of hairs, and amount of pigment of the iris. It is possible to have the factors segregated, and they may occur in any combination.

Sex linkage

Because of the lack of space and equipment, it was not possible to segregate the offspring of all the fish until they had reached maturity in order to determine the sex. However, the evidence from table 1 would indicate that the factors for crescent and twin spot markings are carried by autosomal genes.

Platypoecilus and Lebistes, both belonging to the same sub-family, Poeciliinae, show different types of sex linkage. WINGE proved that Lebistes shows the XY type, while BELLAMY showed that Platypoecilus has the WZ type; that is, the female is heterogametic for sex, while in the XY

type the male is heterogametic for sex. AIDA, in his studies of inheritance in *Aplocheilus latipes*, another small fish of the order Cyprinodontes, showed that the XY type of inheritance held here, and further that a gene for red body color (*R*) was carried both by the X and Y chromosomes. He found, also, that crossing over of the *R* gene occurred between the X and Y chromosomes in the offspring of the backcrosses between heterozygous brown or red males and white females, and also matings of heterozygous red males with heterozygous red females. WINGE found in *Lebistes reticulatus* that the Y chromosome carried recessive genes. FRASER and GORDON found in *Platypoecilus maculatus* the WZ type of inheritance, and showed that the W chromosome usually bears only recessive genes.

There are two possibilities to consider if the WZ type of inheritance functions in Xiphophorus, providing that the factors *C* and *P* are sex-linked.

Case 1—If the W chromosome carries the recessive genes or none.

Case 2—If the W chromosome carries the dominant genes.

In table 1, the parents have the genotypes *CcPp* and *CcPP* for male and female respectively.

DIAGRAM OF CASE 1

Let the W chromosome be represented by | and carrier of either recessive genes or none as regards *C*.

P	*C c* / *P P* ♀ Crescent	×	*C c* ♂ Crescent	
		↓		
F_1	*C C* / *P P* ♂ Crescent	*C c* / *P p* ♀ Crescent	*C c* / *P P* ♀ Crescent	*c c* / *p P* ♀ Non-crescent

There are no non-crescent males, yet two were obtained in table 1. These could not be obtained if this type of inheritance held unless crossing over took place. There were two males obtained out of a total of 15 animals, and this possibility of crossing over, therefore, seems slight.

CASE 2

The W chromosome carrier of the dominant genes:

P	*c C* / *P P* ♀ Crescent	×	*C c* / *P p* ♂ Crescent	
		↓		
F_1	*c c* / *P p* ♂ Non-crescent	*c C* / *P P* ♂ Crescent	*C C* / *PP* ♀ Crescent	*C c* / *P p* ♀ Crescent

There would be no non-crescent females, yet two were obtained in table 1. The only explanation would be that crossing over took place, which does not seem probable since two non-crescent females were obtained out of a total of 15 animals. Further, if dominant *C* is carried on the W chromosome, all the female offspring should carry this gene, no matter what genes are introduced in the male inheritance, and all the females would have the crescent marking,—the exception being if crossing over between the W and Z chromosomes should take place.

From the results in table 1, it would appear that the WZ type of sex inheritance does not operate here.

If the XY type of inheritance is common to Xiphophorus, there are again two possibilities.

Case 1. If the Y chromosome carries recessive genes or none.
Case 2. If the Y chromosome carries the dominant genes.

DIAGRAM OF CASE 1

P		$\frac{C\ c}{P\ P}$ ♀ Crescent	×	$\frac{C\ c}{P\ p}$ ♂ Crescent
	↓			
F_1	$\frac{C\ C}{P\ P}$ ♀ Crescent	$\frac{C\ c}{P\ P}$ ♀ Crescent	$\frac{C\ c}{P\ p}$ ♂ Crescent	$\frac{c\ c}{P\ p}$ ♂ Non-crescent

There were, however, two non-crescent females obtained in this mating, and these again could be accounted for only if crossing over took place, which is not very probable, since two females were obtained out of 15 animals. Also, table 6 indicates that this type of inheritance, as regards these factors, does not hold here. A crescent male mated with a non-crescent female gave all crescent offspring. If there were a sex-linked character, and the Y chromosome a carrier of recessive genes or none, half of the offspring should be non-crescent as the diagram below indicates.

P		$\frac{C\ c}{P\ P}$ ♂ crescent	×	$\frac{c\ c}{P\ P}$ ♀ non-crescent
	↓			
F_1		$\frac{C\ c}{P\ P}$ ♀ crescent		$\frac{c\ c}{P\ P}$ ♂ non-crescent

To obtain all crescent offspring, the *C* factor would have to be carried in the Y chromosome.

CASE 2

If the Y chromosome is a carrier of the dominant genes.

P		*C c* / *P P* ♀ Crescent	×	*c C* / *P P* Crescent	
F_1	*C c* / *P p* ♀ Crescent	*c c* / *P p* ♀ Non-crescent		*C C* x / *P P* ♂ Crescent	*c C* / *P p* ♂ Crescent

In this case, there would be no non-crescent males, two of which were obtained out of 15 animals. Further, all the males would transmit this gene for crescent, and all males would be crescent, despite the gene introduced by the females which is not true.

None of the possible cases of sex inheritance agrees with the results obtained, and it seems evident that the factors for crescent and twin spot marking are carried by autosomal genes.

SUMMARY

The results of the matings of the *Xiphophorus helleri*, variety *rachovii*, in respect to crescent and twin spot markings indicate that:

1. The factor for crescent marking is not carried by non-crescent parents as is proven by series 12.
2. The crescent marking is inherited as a dominant factor.
3. The factor for crescent marking is carried by an autosome. It is, therefore, not sex-linked.
4. Crescent marking is produced by the interaction of two factors *C* and *P*.
5. Twin spot marking is produced by the interaction of two factors, *C* and *p*.
6. Non-crescent or non-spot fish differ as regards the *P* factor. As regards the genotypes of these fish, these are *ccpp*, *ccPP*, and *ccPp*.

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INHERITANCE OF AGE AT SEXUAL MATURITY IN THE DOMESTIC FOWL[1]

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Received January 18, 1934

THE PROBLEM

Dr. RAYMOND PEARL (1912) was the first worker to suggest that sex-linked factors were involved in the inheritance of fecundity. PEARL used as a measure of fecundity the eggs produced during the winter period. He proposed that two major dominant factors were responsible for high egg production, one of which resided in the sex chromosome. GOODALE (1919) and HURST (1921) each failed to confirm the earlier statement of PEARL that a sex-linked factor was involved in the inheritance of fecundity.

GOODALE (1919) called attention to the inadequacy of PEARL's method of attacking the problem of the inheritance of fecundity. He pointed out that there were a number of more or less independent genetic factors responsible for high egg production and that PEARL's method of considering as a unit the total number of eggs produced could not be expected to yield critical results. GOODALE (1922) held that the annual egg record was dependent upon the combined action of the following five characteristics: maturity, intensity, broodiness, persistency, and winter pause.

HAYS (1924) proposed that maturity (age at sexual maturity) was dependent upon two pairs of factors, one of which was sex-linked. He held that the early maturity factor was dominant in each case. The data presented in support of the theory are rather meager. HAYS has suggested that since PEARL's study of inheritance of fecundity utilized only egg production during the winter period, he was probably considering primarily age at sexual maturity. It is readily seen that differences in age at which birds started to lay would materially influence the number of eggs produced in that period.

PUNNETT (1930) outlined what he considered a crucial test for the theory of PEARL that sex-linked factors were involved in the transmission of high fecundity. It had been shown previously that the two genes, silver-gold and barred-non-barred were located on the sex chromosome about 45 units apart (AGAR 1924, SEREBROVSKY and WASSINA 1927, WARREN 1928). PUNNETT stated that if these two genes and the one for fecundity were all on the sex chromosome, through segregation some evidence for linkage among them should be found. In following the segregation of these

[1] Contribution No. 79 from the Department of Poultry Husbandry.

three factors through a male heterozygous for all three, he found no evidence for linkage. His conclusions were based upon the mean egg production of birds segregating for the silver-gold and barred-non-barred pairs of factors. Since he found no pronounced difference in the mean egg production of the various color segregates, PUNNETT concluded that there was no evidence for sex-linked factors influencing egg production. In view of the rather general acceptance of the opinion that the inheritance of the egg production is too complicated to consider the annual record as a unit, PUNNETT's method of attack appears somewhat obsolete. This is especially true when we consider the fact that the only characteristic which has been proposed to show sex-linked inheritance is age at sexual maturity. Even though age at sexual maturity were sex-linked the influence of this factor on the annual record could easily have been obscured by the other factors influencing egg production, especially in view of the small numbers with which he dealt.

EXPERIMENTAL RESULTS

Selection

During the six-year period, 1926–1931, selection was carried out on Single Comb Rhode Island Reds at the KANSAS AGRICULTURAL EXPERIMENT STATION for the establishment of strains differing in age at which they reach sexual maturity. Very little new stock was obtained during the period of the experiment so that the flock remained relatively homogeneous. Age at sexual maturity was measured by the age attained at the laying of the first egg. This limited to females the classification with regard to age at sexual maturity since no satisfactory measure was found which would apply to both sexes. In carrying out selection a somewhat inaccurate estimate was made of the age at sexual maturity in the males, being based upon the plumage and comb development at six months of age. This classification of the males was not used in measuring the progress of selection but probably did increase the effectiveness of selection.

Throughout the period of the experiment care was exercised to house the pullets early enough to obtain an accurate record of the age at first egg. Most of the pullets were hatched between February 15 and April 15 of each year thus avoiding the influence of wide seasonal differences on maturity. The young stock during this period was relatively free from diseases and parasites which might influence the age of attainment of sexual maturity. It is realized, however, that even under the best conditions the health of the bird will influence the onset of production and is probably a source of considerable error which could not be entirely avoided. The pullets were closely observed during the early part of the laying year and any bird showing an inclination to lay eggs outside the trapnests was

eliminated from consideration because of likelihood of error in the record as to date of starting.

During the 1926 season six matings were made for the study of maturity, three being selections for later maturity and three for earlier maturity. In the selection for early maturity, early maturing females were mated to males from early maturing mothers. In each case care was exercised to use for the most part individuals from early averaging families. The same rule held for the late maturity matings and as selection progressed the use of individuals of proved breeding qualities was stressed.

In the selection of birds to be used for continuation of the early and late maturity strain, no individual maturing at an age of more than 225 days was used in the early strain and none having maturity age of less than 235 days was considered for the late strain. The difference in maturity age for the females used in the two strains was usually much greater than the limits mentioned.

Although the data recorded in table 1 show the daughters of the late maturity matings in 1926 to have a mean age of 7.2 days greater than

TABLE 1

Selection for early and late maturity in Rhode Island Reds.

YEAR	EARLY MATURITY STRAIN		LATE MATURITY STRAIN		DIFFERENCE IN MATURITY AGE IN DAYS	DIFFERENCE p.e.
	NUMBER OF DAUGHTERS	MEAN MATURITY AGE IN DAYS	NUMBER OF DAUGHTERS	MEAN MATURITY AGE IN DAYS		
1926	89	269.0±2.00	85	276.2±1.70	7.2±2.6	1:2.8
1927	96	230.8±2.70	84	249.5±3.29	18.7±4.3	1:4.3
1928	73	221.9±3.55	97	247.6±2.85	25.7±4.6	1:5.6
1929	92	211.5±3.13	78	252.3±3.84	40.8±5.0	1:8.2
1930	20	216.7±4.65	29	249.3±4.38	32.6±6.4	1:4.9
1931	45	222.2±5.13	71	269.0±3.37	46.8±6.1	1:7.7

those from the early maturity mating, the difference was not sufficiently large to have statistical significance. During the second year (1927) there was a much better opportunity to apply the progeny test within the late and early maturity strains. This resulted in a greater different in the mean age at which the two strains matured. The 96 female offspring of the four early maturity matings had a mean age at the time of laying of the first egg of 230.8 days while 84 pullets from the three late maturity matings began laying at a mean age of 249.5 days. The difference in age is 18.7 days or 4.3 times its probable error.

After the third year of selection (1928) there was very little change in the mean maturity age of either strain. In fact there was very little change secured in the late maturity strain, practically all of the difference between the two strains resulting from the reduction of the mean age at laying in the early maturity strain.

During 1930 and 1931 when it became evident that further change in the maturity of the two strains was not probable, an increasing portion of the facilities was given over to the crossing of the two strains. This accounts for the reduction in number of females tested in the two strains during the last two years. By this time it seemed to be well demonstrated that a heritable difference in age at sexual maturity had been brought about by selection. The next step undertaken was to analyze the mode of inheritance of the factor or factors involved.

Recriprocal matings of the early and late maturity strains

During the 1930 and 1931 breeding seasons reciprocal matings were made between the early and late maturing strains. A comparison of the age at sexual maturity of the female offspring of reciprocal matings should indicate whether sex-linked factors influenced the age at which pullets started to lay. An equal number of pens were headed by males from the late and early maturity strains and each pen carried females not only from the strain to which the male belonged but also from the other strain. By this method each male was checked upon by matings within his own strain at the same time that he was being out-crossed to females of a different maturity age.

It chanced during the first year (1930) in which crosses were made between the early and late maturity strains, that the difference between the pure early and late strain matings was less than those for the preceding or succeeding years. There were two matings of early maturity males by late maturity females and three of late maturity males by early maturity females. The 31 female offspring (table 2) from the former mating had a

TABLE 2

F_1 generation results from crossing early and late maturity strains of Rhode Island Reds.

	EARLY MATURITY MALE BY LATE MATURITY FEMALE				LATE MATURITY MALE BY EARLY MATURITY FEMALE			
YEAR	MALE BAND NUMBER	NUMBER OF MATES	NUMBER OF DAUGHTERS	DAUGHTERS' MEAN MATURITY AGE	MALE BAND NUMBER	NUMBER OF MATES	NUMBER OF DAUGHTERS	DAUGHTERS' MEAN MATURITY AGE
1930	1082M	4	28	221.8± 2.69	1081M	1	4	245.8±9.57
	1088M	1	3	231.3±10.70	1083M	3	20	247.0±4.57
					1084M	3	18	214.3±5.56
Total and mean			31	222.7±2.66			42	232.9±3.75
1931	1086M	1	11	200.8± 8.57	1083M	2	10	223.6±9.39
	1128M	3	13	230.8± 8.87	1130M	2	7	245.7±12.19
	1131M	2	12	219.7± 9.67	1132M	4	34	231.5±4.80
					1133M	3	29	268.7±6.76
Total and mean			36	217.9± 5.42			80	244.8±3.83

mean age of 222.7 days at the laying of the first egg while the latter mating produced 42 daughters that had a maturity age of 232.9 days. If sex-linked factors influenced the age at sexual maturity it would be expected that the female offspring of the latter type of mating would show later maturity than those of the former. The difference (10.2 ± 4.59) is in the expected direction but its probable error is too large for it to have much significance.

The second year's (1931) results were much more definite. The 36 daughters of the mating of the early maturity male by late maturity female had a mean age of 217.9 days and the reciprocal cross produced 80 female offspring which had a maturity age of 244.8 days. The difference between the two types of matings was 26.9 days which was 4.1 times its probable error. The fact that the mating of males from the early maturity strain to females from the late maturity strain produced daughters starting to lay at an earlier age than did those from the pure early maturity mating for that year is of interest. The age at sexual maturity for these cross-strain females was very similar to the pure early-strain birds during the last few years of selection. This would indicate that the major factorial difference was sex-linked or that any autosomal factors for late maturity must be completely recessive. The results for the mating of late maturity males to early maturity females were not so similar to those from the pure late maturity matings. This mating produced daughters which had a mean age of 244.8 days at the laying of the first egg while the pure late maturity matings for the same season gave daughters with a mean maturity age of 269 days. These results would tend to support the view that autosomal factors were also involved and that the influence of the autosomal and sex-linked factors were cumulative. The intermediate maturity here may be interpreted as a result of the conflicting effects of the sex-linked and autosomal factors. HAYS has suggested that both autosomal and sex-linked factors are involved in the inheritance of age at sexual maturity and in referring to the early maturity factor stated that "either combined or alone, simplex or duplex, these factors produce a bird that normally begins to lay at 215 days or less." A comparison of the results given in table 2 would indicate that a female carrying the sex-linked factor for late maturity (and having only one sex chromosome she can only carry it in a single dose) and being heterozygous for the autosomal factor or factors, matures somewhat earlier than one having the same sex chromosomal constitution and being homozygous for late maturity. Thus it would appear that the expression of the early maturity gene is in certain combinations dependent upon the individual's constitution with regard to the other genes influencing maturity age.

Backcross generation

The backcross results further substantiate the conclusions drawn from the F_1 generation results. Two types of matings were made and in each case the male was from the early maturity strain and was mated to females from the reciprocal F_1 crosses. If we assume that a sex-linked and an autosomal factor are involved, then the one type of F_1 generation female used would have the constitution $E\ E'e'$ and the other the constitution $e\ E'e'$. In the foregoing use of symbols (E) is the sex-linked factor and (E') the autosomal factor. The 132 female offspring from the backcross of F_1 generation females from the cross of early maturity males by late maturity females, by males from the early maturity strain, had a mean maturity age of 214.4 ± 2.17 days. F_1 generation females (249 birds) from the reciprocal cross when backcrossed to males from the early maturity strain had a mean maturity age of 217.5 ± 1.54 days. There was found to be no significant difference between the two types of backcrosses. The maturity age obtained from these matings is very similar to that characteristic of the pure early maturity strain. The duplication here of the mean of the pure early maturity strain substantiates the conclusion drawn from the F_1 generation that when a female carries the sex-linked factor for early maturity it makes no difference whether she is heterozygous or homozygous for the autosomal factor for early maturity. In each type of backcross half the female offspring would be homozygous for the autosomal factor for early maturity and half heterozygous for this factor. If the effect of the autosomal factor had been different in a homozygous or heterozygous condition when in combination with the sex-linked early maturity factor, the mean age here should have been greater than for the pure early strain and there should have been a bimodal shape to the distribution curve for the female offspring in the backcross generation. The maturity ages of the female offspring from the larger (backcross of female from cross of late male by early female) of the two backcross series are presented in the form of a distribution in the following table:

1. Maturity age—170 days or less	5
2. Maturity age—171–190 days	47
3. Maturity age—191–210 days	67
4. Maturity age—211–230 days	71
5. Maturity age—231–250 days	24
6. Maturity age—251–270 days	12
7. Maturity age—271–290 days	8
8. Maturity age—291–310 days	8
9. Maturity age—311 days or more	7
Total	249

There is here no evidence of any bimodality. The curve would not descend rapidly in the later maturity classes but this increase in number of the slowest maturing birds is probably due to factors other than genetic.

The results of the backcross and F_1 generation support the hypothesis of HAYS in indicating that both sex-linked and autosomal factors are involved in determining the age at which birds reach sexual maturity. The results obtained in the F_1 generation indicate rather strongly that HAYS was in error in believing that either the sex-linked or the autosomal factor could when acting alone produce early maturity. No birds were obtained in which the sex-linked early maturity factor was acting alone but the cross of a late maturity male to early maturity females produced female offspring of the constitution *e E′e′* which had a mean maturity age of 244.8 days while pure early maturity females (*E E′E′*) matured in 222.2 days

TABLE 3

Comparison of maturity age of daughters of individual males when mated to females of their own strain and to females of differing maturity age.

YEAR	MALE BAND NUMBER	LATE MATES		EARLY MATES	
		NUMBER OF DAUGHTERS	DAUGHTERS' MEAN MATURITY AGE	NUMBER OF DAUGHTERS	DAUGHTERS' MEAN MATURITY AGE
	Late males				
1930	1081M	11	244.4± 6.5	4	245.8± 9.6
	1083M	6	258.8± 8.2	20	247.0± 4.6
	1084M	12	249.0± 7.5	18	214.3± 5.6
	Total and mean	29	249.3± 4.4	42	232.9± 3.8
	Early males				
	1082M	28	221.8± 2.7	9	219.7± 5.5
	1088M	3	231.3±10.7	11	214.2± 7.1
	Total and mean	31	222.7± 2.7	20	216.7± 4.7
1931	Late males				
	1083M	21	268.5± 6.0	10	223.6± 9.4
	1130M	8	278.9±10.9	7	245.7±12.2
	1132M	36	261.8± 4.5	34	231.5± 4.8
	1133M	6	296.0±12.2	29	268.7± 6.8
	Total and mean	71	269.0± 5.1	80	244.8± 3.8
	Early males				
	1086M	11	200.8± 8.6	19	228.9± 8.4
	1128M	13	230.8± 8.9	20	201.8± 3.9
	1131M	12	219.7± 9.7	6	269.0±18.1
	Total and mean	36	217.9± 5.4	45	222.2± 5.1

and pure late maturity females (*e e′e′*) matured in 269.0 days. This tendency is noted from the data in table 3 where a comparison is made of the results from individual males when mated to both early and late maturing females. The results of the two generation crosses seem to give the following mean maturity ages for the various genotypes in the females.

E E′ E′—222 days maturity
e e′ e′—269 days maturity
E E′ e′—217 days maturity
e E′ e′—245 days maturity

From these results it may then be concluded that the sex-linked early maturity factor in combination with the autosomal factor either in a homozygous or heterozygous condition produces early maturity individuals. The autosomal factor for early maturity when in a heterozygous condition and in combination with the sex-linked factor for late maturity gives intermediate maturity. Since maturity age cannot accurately be measured in males it cannot be stated what influence the sex-linked early maturity factor would have in a heterozygous condition. It is realized that the backcross data do not provide a crucial test for the existence of sex-linked factors for age at sexual maturity. Since we are limited to the female sex in classifying birds for maturity age no backcross or F_2 generation cross will provide such a test. We must, therefore, be satisfied with evidence from the F_1 generation and the fact that the later generation results do not contradict this interpretation.

TABLE 4

Distribution of maturity age among males resulting from various types of matings between early and late maturity strains.

	NUMBER OF MALES	PERCENTAGE OF EARLY MATURITY	PERCENTAGE OF MEDIUM MATURITY	PERCENTAGE OF LATE MATURITY	RATIO OF EARLY TO LATE	CONSTITUTION OF MALE OFFSPRING
Early ♂×early ♀	52	19.2	69.2	11.5	1:0.60	EEE′E′
Late ♂×late ♀	46	8.7	60.9	30.4	1:3.49	eee′e′
Late ♂×early ♀	96	33.3	46.9	19.8	1:0.59	EeE′e′
Early ♂×late ♀	41	17.1	73.2	9.8	1:0.57	EeE′e′
Backcross of F_1 ♀ (early ♂×late ♀) to early ♂	61	13.1	70.5	16.4	1:1.25	EEE′E′ EEE′e′
Backcross of F_1 ♀ (late ♂×early ♀) to early ♂	91	20.9	60.4	18.7	1:0.89	EeE′E′ EeE′e′

Maturity in males

In table 4 are recorded the data on inheritance of age at sexual maturity in males. As was earlier stated the estimation of the age at sexual maturity in males is subject to considerable error. The results are given here only because of the value they have in supplementing the data on females and due to already mentioned analytical difficulties arising from being limited to the classification of one sex.

In obtaining these data males reared from the maturity study matings were segregated by hatches when they were approximately six months

old and graded as early, medium, and late. Judgment was based upon plumage and comb development. Although the method of classification is somewhat crude and subject to considerable error, the data supply some evidence as to the mode of inheritance of age at sexual maturity that cannot be obtained in any other manner. In table 4 the data are recorded in percentage of those falling in the three groups, early, medium, and late. The ratio of those of early classification to those in the late group is also given for each type of mating.

The ratio of 1:0.60 obtained in the early by early mating is very similar to those (1:0.59 and 1:0.57) obtained for F_1 generation males from reciprocal crosses. Since the males from these two latter matings were heterozygous for the sex-linked and autosomal factors (*EeE'e'*) it would seem that when the two factors for early maturity are present it makes little difference whether they are in a heterozygous or homozygous condition. Since the standard for classification was somewhat indefinite it may not be well to attempt to compare the first four matings listed in table 4 with the two backcrosses since the former were made in 1931 while the latter two were carried out in 1932. The evidence from the two backcrosses is not entirely consistent with the other data. The male offspring from the two matings differ only in the fact that the first listed ones are homozygous for the sex-linked factor while the latter ones are heterozygous for this factor. The first mating gave a lower percentage of early maturing sons than did the latter. This difference is not to be expected from the genetic constitution of the two groups of males. From these results it can at least be said that we have here confirmatory evidence for the earlier stated conclusions that the sex-linked factor has the same effect in a heterozygous condition that it has in a homozygous state.

DISCUSSION OF RESULTS

The results for the study of maturity in both female and male Rhode Island Reds would tend to substantiate the findings of HAYS that there are both sex-linked and autosomal factors involved in the inheritance of age at sexual maturity. The data also support the view that the sex-linked factor for early maturity is completely dominant and has the same effect regardless of the constitution with respect to the autosomal factor. There is also evidence that the autosomal factor for early maturity is not completely dominant when acting in the presence of the sex-linked late maturity. For simplicity of treatment the results have been analyzed as if there were only one pair of sex-linked and one pair of autosomal factors involved. It is realized that the results do not exclude the possibility of several pairs of factors having an influence upon maturity age but the evidence supports the view that at least some sex-linked and autosomal factors are concerned.

LOCATION OF THE GENE FOR AGE AT SEXUAL MATURITY ON THE SEX CHROMOSOME

Evidence for sex linkage of maturity differences in crossbreeding

The foregoing studies on Single Comb Rhode Island Reds have indicated that differences in age at sexual maturity in this breed were inherited in a sex-linked manner. The Mediterranean breeds are known to mature at an age considerably less than that found in the heavier dual purpose breeds. It is then of interest to determine whether the difference in age at sexual maturity among these breeds is due to sex-linked factors.

WARREN (1930) made reciprocal crosses between Single Comb White Leghorns and Single Comb Rhode Islands Reds. The hybrids were half-sisters to the purebreds with which they were being compared. The 51 White Leghorns had a mean maturity age of 172.4 days and the 49 Rhode Island Reds 247.6 days. The cross of a White Leghorn male by a Rhode Island Red female produced 58 hybrid females maturing at the average of 175.9 ± 2.27 days while the reciprocal of this mating produced 52 daughters with a mean maturity age of 206.4 ± 3.78 days. Since the White Leghorns had a much earlier maturity age than the Rhode Islands Reds the difference between the maturity ages of reciprocal crosses is in the expected direction provided sex-linked factors are responsible for the difference.

A second comparison was made of the maturity age of hybrids from reciprocal matings of White Leghorns and Rhode Island Reds. In this case the Rhode Island Red stock came from the late maturity strain which had a mean age at maturity of 249 days for that year (1930). The particular females used in the mating with the White Leghorn males had a mean age at maturity of 269 days. The White Leghorn stock used had a mean maturity age of 175 days for that year. The 77 female offspring from the cross of the White Leghorn male by Rhode Island Red females matured at the mean age of 197.7 ± 2.94 days while the 171 daughters of the reciprocal cross, Rhode Island Red male by White Leghorn females, had a mean maturity age of 209.6 ± 2.18 days. Here again the earlier maturing hybrids came from the cross in which the father was from the early maturity stock. The actual difference in maturity age was 11.9 days which was 3.26 times the error of the difference. In both the crosses cited here it is seen that statistically significant differences were found between the reciprocal crosses and in each case the difference was in the expected direction provided sex-linked factors were involved. It would, therefore, appear that differences in age at sexual maturity between the Mediterranean and the larger breeds are at least partially due to sex-linked factors.

Order of genes

Some attention has already been given to the mapping of the sex-linked factors in the fowl (SEREBROVSKY 1927, WARREN 1928, HERTWIG and RITTERSHAUS 1929, and MACARTHUR 1933). The best known sex-linked factors are gold-silver, rate of feathering, barring, and shank color. Workers are in fair agreement by finding 10 to 19 percent of crossing over between the genes for gold-silver color and rate of feathering. The genes for barring and shank color have shown themselves to be only loosely linked with either gold-silver or rate of feathering (crossing over percentage 40–48). No very satisfactory data have been published on the linkage relations of shank color and barring but their reaction with other factors in the sex chromosome would indicate that they are closely associated.

The writer (1928) tentatively proposed the order of the genes on the sex chromosome to be barring-silver-rate of feathering. Since both silver and rate of feathering show very loose linkage with barring it remained a question as to the exact order of these two factors. In the 1928 publication the crossover value for the rate of feathering and barring was 48 and for silver and barring 46. In table 5 are presented two additional years' results on the linkage relations of this pair of sex-linked factors. Some inconsistencies are seen in the two members of the non-crossover and crossover classes but these can probably be attributed to the difficulty of recognition of the phenotypes when both barring and gold are involved. Gold is not well expressed in a barred bird carrying extended black and the barring factor has little opportunity of expression when the black is restricted to the degree that it is in the Columbian pattern. In the latter group evidence of the existence of the barring factor was usually found in the feathers of the hackle but in some cases the black area was so restricted that decision was difficult. This fact is probably responsible for the shortage of birds in the barred class. In the barred birds carrying extended black (Barred Plymouth Rock pattern) the presence of gold was not easily recognized. If such individuals showed any buff or red in the neck or breast region they were classified as gold, but in the absence of these colors, were called silver. The difficulty of recognizing gold and silver when in combination with barring and other pattern factors resulted in some variations in the ratio of these colors in the different classes. The two years' results given in table 5 and the writer's previously published results are all in agreement in indicating a higher percentage of crossing over between barring and rate of feathering than between barring and gold-silver. These results would tend to confirm the view that although both rate of feathering and gold-silver are widely separated from barring, the latter (gold-silver) is located between rate of feathering and barring.

TABLE 5

Linkage relations of the sex-linked factors barring with gold-silver and with rate of feathering.

FACTORS	NON-CROSSOVERS			CROSSOVERS				
BARRING AND GOLD-SILVER	NON-BARRED GOLD	BARRED SILVER	TOTAL	NON-BARRED SILVER	BARRED GOLD	TOTAL	GRAND TOTAL	PERCENTAGE CROSSING OVER
Brown Leghorn by Barred Rock	51	81	132	46	60	106	238	44.5
White Leghorn by Rhode Island Red	203	59	262	69	123	192	454	42.3
BARRING AND RATE OF FEATHERING	NON-BARRED EARLY	BARRED LATE	TOTAL	NON-BARRED LATE	BARRED EARLY	TOTAL	GRAND TOTAL	PERCENTAGE CROSSING OVER
Brown Leghorn by Barred Rock	51	76	127	52	65	117	244	48.0
	NON-BARRED LATE	BARRED EARLY	TOTAL	NON-BARRED EARLY	BARRED LATE	TOTAL	GRAND TOTAL	PERCENTAGE CROSSING OVER
White Leghorn by Rhode Island Red	59	36	95	50	39	89	184	48.4

Additional evidence was obtained from the 1931 data which involved rate of feathering, silver and barring in the same cross. They were as follows: 25 early feathering, silver, barred; 48 late feathering, gold, non-barred; 9 early feathering, gold, barred; 3 late feathering, silver, barred; 24 early feathering, silver, non-barred; 24 early feathering, gold, non-barred; 3 late feathering, silver, non-barred; and 33 late feathering, gold, barred. If we assume that silver is located between rate of feathering and barring, then breaks between barring and rate of feathering should frequently affect the relationships of silver and rate of feathering. It would also be true that breaks between silver and rate of feathering would not affect the relationships between silver and barring except in cases of double crossing over where breaks occurred simultaneously between silver and barring and silver and rate of feathering. In 84 breaks between barring and rate of feathering, 27 were accompanied by changes in the relationships of silver and rate of feathering. There were only 12 out of 69 cases where the relationships of silver and rate of feathering were affected by breaks between barring and silver. Likewise, if silver occupied the end position having rate of feathering between it and barring, breaks between silver and rate of feathering should not affect the relationships of rate of feathering and barring. A summarization of the breaks between silver and rate of feathering revealed the fact that in a total of 39 such breaks, 27 also changed the relative positions of rate of feathering and barring. The only way to account for these 27 cases is by double crossing over and it seems improbable

that the percentage would be so high. Therefore, all the results seem to indicate strongly that silver is located between barring and rate of feathering. It should be noted that this arrangement of the genes agrees with the findings of SEREBROVSKY and WASSINA (1927) but not with the conclusions of MACARTHUR (1933).

Position of sex-linked gene for maturity

Having accumulated evidence that the differences in age at sexual maturity both in Rhode Island Reds and between Rhode Island Reds and White Leghorns are inherited in a sex-linked manner, it is of interest to determine the linkage relations between the responsible gene or genes and the other known genes in the sex chromosome. The method followed is similar to that made use of by PUNNETT (1930) except that consideration is here given to age at first egg instead of annual record. The sex-linked factor for age at sexual maturity should show linkage with the factor with which it is most closely associated on the sex chromosome. If early maturity and silver plumage were characteristic of a breed, and the two factors were closely associated on the sex chromosome, then there should be a tendency for these two characters to be associated in segregation. In other words, if the early maturity silver stock was mated to a late maturity gold stock, the silver segregates should be of earlier maturity than the golds. If the golds and silvers should have similar maturity then it could be assumed that the genes for gold-silver and for maturity age were not closely associated on the sex chromosome. Then if several known sex-linked genes were involved at the same time the age at maturity of the segregates should indicate the approximate location of the gene for age at sexual maturity.

The Single Comb Brown Leghorn and the Barred Plymouth Rock breeds were chosen for the first test for the linkage relations of the factor for age at sexual maturity. These two breeds were crossed because they produced a hybrid male heterozygous for three sex-linked factors, barred, rate of feathering, and gold-silver. The Brown Leghorn carried the recessive gold, recessive early feathering, and recessive non-barring. The Barred Plymouth Rock carried the three dominant alleles. It was also known that there was the characteristic difference in age at sexual maturity between these two representatives of the Mediterranean and American classes, the Brown Leghorn having the earlier maturity. It would have been desirable to have known the average difference in age at sexual maturity between these two breeds but since the available stock of each was limited this was not feasible.

A Brown Leghorn male was mated to three Barred Plymouth Rock females for the production of males heterozygous for the four sex-linked

factors, rate of feathering, barring, gold-silver, and age at sexual maturity. Since age at sexual maturity could be measured only in the females and since the female bird has only one sex chromosome, it made little difference what type of females were mated to the multiple heterozygotes. The female offspring having only one sex chromosome and receiving it from their sire only, must show the sex-linked constitution of the gamete which they received from him.

Six Brown Leghorn-Barred Plymouth Rock hybrid males were mated to a group of 70 Rhode Island Red females. Females of the Rhode Island Red breed were chosen primarily because of their availability. The heterozygous males received early feathering, gold, non-barred, and early maturity in one gamete and late feathering, silver, barred, and late maturity in the other. Therefore, the allelic pair of characters which shows the greatest difference in maturity between its segregating members should indicate the approximate location of the gene for age at sexual maturity.

In table 6 are presented comparisons of the mean age at sexual maturity for the members of the three sex-linked pairs of factors in the Brown Leghorn-Barred Rock cross. There was very little difference in maturity age for the early and late feathering females, the 146 late feathering pullets having a mean age of 194.4 ± 2.17 days and for the 149 early feathering ones, 193.8 ± 1.92 days. When classified for gold and silver plumage the difference was also of no significance, the silver ones maturing in 192.4 ± 2.14 days and the golds in 195.5 ± 1.95 days. The only case where the difference is at all great is for the barred and non-barred groups. The barred birds had a maturity age of 200.7 ± 2.31 days while the non-barred ones had a mean maturity age of 189.5 ± 1.80 days. The difference of 11.2 days in age at sexual maturity of the barred and non-barred groups approaches statistical significance and emphasized the desirability of a repetition of the experiment.

In order to make the results more critical the experiment was repeated with breeds of fowl concerning which there was a more dependable knowledge of the age at sexual maturity. The two breeds used were the Single Comb White Leghorn and Single Comb Rhode Island Red. White Leghorn males from a strain with a mean maturity age of 175 days were mated to Rhode Island Red females which came from a strain that had been selected for a period of years for late maturity (mean maturity age 249 days). The F_1 generation results from reciprocal matings between these two strains have been given under the discussion on evidence for sex linkage of maturity age in crossbreeding. The data indicated that sex-linked factors were involved. The chief reason that this mating was not originally used was the fact that the presence of dominant white in the White Leghorn interfered with the expression of barring. This fact neces-

sitated the discarding of one-half of the offspring in the backcross generation.

The F_1 generation males from the above mentioned cross were heterozygous for barring, silver, rate of feathering, maturity age, and dominant white. The combination of barring, silver, early feathering, early maturity was brought in from the White Leghorn and non-barring, gold, late feathering, and late maturity from the Rhode Island Red breed. The White Leghorn carries barring and silver in a hypostatic condition. Previous observations had demonstrated that the two strains used were homozygous for the two types of feathering.

The F_1 males were mated to Rhode Island Red females and the female offspring were classified for barring, gold-silver, rate of feathering, and age at sexual maturity. As has been mentioned already it was necessary to discard all white chicks produced in this mating because of the dominant white masking the expression of barring and gold-silver. Since dominant white was known to be an autosomal factor, the elimination of the white chicks should in no way effect the segregation of the sex-linked factors. Since half of the chicks were discarded because of white and since only females could be classified for age at sexual maturity, only one-fourth of the chicks hatched were of any value. Allowing for the mortality normally occurring before maturity, it was necessary to hatch about 900 chicks to obtain a test for maturity on 186 females.

TABLE 6

Comparison of mean age at sexual maturity of allelic pairs in the case of three sex-linked factors. Differences in maturity age should indicate the approximate location of the maturity gene on the sex chromosome.

ALLELES	BROWN LEGHORN—BARRED ROCK CROSS		WHITE LEGHORN—RHODE ISLAND RED CROSS	
	NUMBER OF FEMALES	MEAN AGE AT SEXUAL MATURITY	NUMBER OF FEMALES	MEAN AGE AT SEXUAL MATURITY
Late feathered	146	194.4±2.17	89	215.5±2.71
Early feathered	149	193.8±1.92	82	198.8±2.68
Silver	147	192.4±2.14	62	189.4±2.70
Gold	148	195.5±1.95	120	217.5±2.25
Barred	121	200.7±2.31	75	208.3±3.10
Non-barred	174	189.5±1.80	109	207.3±2.28

The data in table 6 under the heading White Leghorn-Rhode Island Red cross are from this experiment. In the pair of factors responsible for the barred or non-barred condition there was no significant difference in age at sexual maturity of the two groups of segregates. The 75 barred females had a mean maturity age of 208.3 days and the non-barred ones,

207.3 days. The original combination was early maturity with barring and late maturity with non-barring. The very slight difference is in the direction opposite to be expected and, therefore, would indicate that the factors for barring and for age at sexual maturity were not closely associated on the sex chromosome. Since the barring factor is widely separated from the factors for rate of feathering and for gold-silver and not closely associated with the factor for age at sexual maturity, it might be expected that there would be linkage between the latter factor and those for rate of feathering and gold-silver. Since the parental combinations were early maturity and silver, and early maturity and early feathering, it would be expected that the alleles of silver and early feathering would show later maturity. The results given in table 6 show the silver females to have matured 28 days earlier than did the gold ones and the early feathering females to have started to lay 17 days earlier than did the late feathering ones. The difference in the first case is 8 times its probable error and in the second 4.4 times its probable error. These differences are statistically significant and would, therefore, indicate that the factor for age at sexual maturity is located on the sex chromosome somewhere between those for rate of feathering and barring. The results here are more significant when we consider the fact that linkages of the maturity factor with three known points on the sex chromosome are in agreement. The difference in results between this experiment and the one using the cross of the Brown Leghorn and Barred Rock is difficult to account for unless the former cross did not involve sex-linked factors influencing maturity. In the former case differences in age at sexual maturity were assumed and not actually proved.

The findings here are also in agreement with the results in table 5 in showing closer linkage with silver-gold than with rate of feathering. The earlier work had indicated that these three genes were located on the sex chromosome in the following order: rate of feathering, gold-silver, and barring. If the proposed order of genes is correct and the factor for age at sexual maturity is located between barring and gold-silver, it would necessarily show closer linkage with gold-silver than with rate of feathering. If the factor for age at sexual maturity were located between gold-silver and rate of feathering, it might also show closer linkage with the former than the latter but since these two factors are themselves fairly closely associated (about 15 units apart), the maturity factor would necessarily need to be very closely linked with each. This does not appear to be true and the order of the genes on the sex chromosome is probably rate of feathering, gold-silver, age at sexual maturity, and barring.

In order to determine approximately the position of the gene for age at sexual maturity on the sex chromosome, calculations were made of the percentage of crossing over between this factor and the other sex-linked fac-

tors. It is realized that in working with a physiological factor of this type considerable error will enter into the calculation. Many environmental factors are known to interfere with the expression of the factor for age at sexual maturity. An additional problem is to establish a dependable dividing line between the late and early maturity groups. For purposes of making the calculation, the dividing line was arbitrarily placed at 205 days, thus segregating the birds into two approximately equal groups. Theoretically, the segregation of the sex-linked factors for maturity should produce equal numbers of early and later maturing individuals. Then in determining the linkage relations for the gene for age at sexual maturity and the gene for gold-silver the non-crossover classes would be silver-early maturity and gold-late maturity and the crossover classes would be silver-late maturity and gold-early maturity. In the case of the relations of age at sexual maturity and rate of feathering the classes are obtained by substituting early feathering for silver and late feathering for gold.

The data for the linkage relations calculated in the manner described are given in table 7. In the measurement of linkage between age at sexual maturity and gold-silver it was found that the percentage of crossing over

TABLE 7

Percentage crossing over between the gene for age at sexual maturity and the sex-linked genes, gold-silver, rate of feathering, and barring.

FACTORS	NON-CROSSOVERS			CROSSOVERS			GRAND TOTAL	PERCENTAGE CROSSING OVER
	SILVER EARLY	GOLD LATE	TOTAL	SILVER LATE	GOLD EARLY	TOTAL		
Age at sexual maturity and gold-silver	65	44	109	38	22	60	169	35.5
	EARLY-FEATH. EARLY	LATE-FEATH. LATE	TOTAL	EARLY-FEATH. LATE	LATE-FEATH. EARLY	TOTAL	GRAND TOTAL	PERCENTAGE CROSSING OVER
Age at sexual maturity and rate of feathering	50	50	100	32	37	69	169	40.8
	BARRED EARLY	NON-BARRED LATE	TOTAL	BARRED LATE	NON-BARRED EARLY	TOTAL	GRAND TOTAL	PERCENTAGE CROSSING OVER
Age at sexual maturity and barring	34	46	80	36	53	89	169	52.7

was 35.5. For rate of feathering and age at sexual maturity the percentage of crossing over was found to be 40.8. The barring factor and age at sexual maturity were found to give 52.7 crossing over, indicating no linkage. No attempt was made to treat statistically the foregoing data since errors of classification were probably much greater than those of random sampling. Although these calculations are considered of little value in determining

the exact location of the gene for age at sexual maturity they do supply some information as to the approximate position of this factor.

These results would support the view that the order of the genes in the sex chromosomes is as follows: rate of feathering, gold-silver, age at sexual maturity, and barring. The available evidence indicates that the gene for age at sexual maturity is located somewhat nearer gold-silver than barring.

SUMMARY

1. By selection it was possible to establish from a flock of fowls, two strains of Single Comb Rhode Island Reds differing considerably (about 40 days) in the mean age at sexual maturity.

2. A genetic analysis indicated that both sex-linked and autosomal factors determined age at sexual maturity in this breed of fowl.

3. Evidence was obtained to show that the difference in age at sexual maturity between the White Leghorn and Rhode Island Red breeds was at least partially due to sex-linked factors.

4. Linkage results from the White Leghorn-Rhode Island Red cross indicate that the sex-linked factor for age at sexual maturity is located between the factor for barring and that for gold-silver.

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